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ABSTRACT OF THESIS

A COMPARATIVE STUDY OF LARVAL GENE EXPRESSION BETWEEN A PAEDOMORPHIC AND METAMORPHIC SPECIES OF AMBYSTOMATID SALAMANDER

Ambystoma tigrinum undergoes an obligatory metamorphosis while *A. mexicanum* fails to metamorphose and exhibits paedomorphosis. While it is clear that salamander paedomorphosis is associated with genetic changes that delay developmental timing, it is not clear when and how these changes manifest during development. It is possible that paedomorphic and metamorphic larvae show equivalent patterns of developmental until late in the larval period, when brain regions become competent to stimulate the release of metamorphic hormones. To test this hypothesis, I compared gene expression patterns between the brains of *A. mexicanum* and *A. t. tigrinum* larvae. In support of the developmental equivalence hypothesis, 114 differentially expressed genes (DEGs) were identified in common between the species and all but 2 showed the same temporal pattern of expression. However, more DEGs were identified uniquely from each species. In particular, several genes that are associated with the hypothalamus-pituitary-interrenal axis, which is implicated in metamorphic regulation in amphibians, exhibited significant expression differences between *A. mexicanum* and *A. t. tigrinum* larvae. The results show that metamorphic and paedomorphic modes of development are associated with different transcriptional programs in the brain and these programs diverge during early larval development.

KEYWORDS: Ambystoma; metamorphosis; microarray; gene expression; brain

Meredith A. Boley

4/28/09

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SALAMANDER

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THESIS

MEREDITH A. BOLEY

THE GRADUATE SCHOOL
UNIVERSITY OF KENTUCKY

2009

A COMPARATIVE STUDY OF LARVAL GENE EXPRESSION BETWEEN A
PAEDOMORPHIC AND METAMORPHIC SPECIES OF AMBYSTOMATID
SALAMANDER

THESIS

A thesis submitted in partial fulfillment
of the requirements for the degree of Master of Science in the
College of Arts and Sciences
at the University of Kentucky

By

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Lexington, Kentucky

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2009

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CHAPTER ONE: INTRODUCTION

Darwin (1859) proposed that evolution by natural selection is a gradual process that results in continuous phenotypic variation among species. However, there are many examples where discontinuous phenotypes are observed among related species and thus appear to evolve rapidly. That evolution could suddenly “leap forward” led evolutionary biologists to extend Darwin’s theory to account for the origin of novel phenotypes. One very old idea is that novel and dramatically different phenotypes originate from mutations of genes that regulate key developmental or physiological processes during ontogeny. In particular, Goldschmidt (1940) proposed that mutations occasionally yield individuals within populations that deviate radically from the norm. He termed these individuals “hopeful monsters”. If the novel phenotypes of hopeful monsters arise under the right environmental circumstances, these will fix and the population may found a new species. While this idea was discounted during the Modern Synthesis, aspects of the hopeful monster hypothesis have been substantiated in recent years. For example, it is clear that dramatic changes in phenotype can occur as a result of few mutational changes of key developmental genes (Carroll, 2000; Gerhart and Kirschner, 1997; Halder et al., 1995; Paigen, 1989; Raff and Kaufman, 1983; Wang et al., 1999). Also, a growing number of studies are showing that phenotypic differences among species often map to relatively few quantitative trait loci (QTLs) and genes (Bergland, et al. 2008; Doebley et al., 1997; Gailing and Bachmann, 2000; Voss and Shaffer, 1997; Wang et al., 1999). These findings are motivating renewed interest in the study of hopeful monsters and the perspectives they can provide about the evolution of genetic and developmental mechanisms (Theissen, 2006, 2009).

At least three lines of evidence led Goldschmidt to cite the Mexican axolotl (*Ambystoma mexicanum*) as one of the original hopeful monsters. First, the axolotl exhibits a very different ontogeny from other closely related tiger salamanders. Whereas many tiger salamanders undergo an obligatory metamorphosis during ontogeny that allows for a transition from an aquatic habitat to a more terrestrial habitat, the axolotl evolved a non-metamorphic life cycle that is often referred to as paedomorphic in the literature. This extreme example of discontinuous phenotypic variation supports a model of evolution by heterochrony: larval morphological traits of ancestral metamorphic forms

are observed in the adult stage of a derived paedomorphic form. In the minds of early evolutionary biologists, these patterns were so clearly supportive of heterochrony that the Mexican axolotl became the exemplar of evolution by neoteny (Kollman, 1885; Gould, 1977a). A second reason Goldschmidt cited the axolotl was physiological - Huxley (1920) had shown that a single molecule – thyroid hormone (TH) - was capable of rescuing metamorphosis in the axolotl. Thus, the axolotl seemed to be an example of evolution waiting around for the right macromutation to happen – simply block a single physiological step in TH regulation and instantly a novel form is originated. A third reason Goldschmidt cited the axolotl was environmental. Previous researchers had noted that the axolotl was endemic to high quality and permanent aquatic habitats of Xochimilco, which is near present day Mexico City (Gadow, 1903). The evolution of paedomorphosis seemingly allowed the axolotl to exploit an empty niche that was originally devoid of predators.

Since Goldschmidt, it is amazing how well the axolotl has withstood the scrutiny of scientific investigation to remain the quintessential hopeful monster. Speculation that the paedomorphic condition of the axolotl could have a simple genetic basis was supported when genetic studies showed that metamorphic and paedomorphic forms could be segregated according to Mendelian expectations (Humphrey, 1967; Voss, 1995). In particular, when *A. mexicanum* from domestic stocks are crossed to the metamorphic *A. t. tigrinum*, the resulting hybrids undergo metamorphosis. Then, when these hybrids are backcrossed to axolotls, there is 1:1 segregation of phenotypes. These crosses show that *A. t. tigrinum* carries a dominant factor that is necessary for metamorphosis. Linkage analysis has mapped this major effect QTL (*met*) to a small interval of the axolotl genome (Voss and Shaffer, 1997). It is important to note that *met* not only associates with the segregation of discrete phenotypes (metamorphosis and paedomorphosis), it also explains variation in metamorphic timing. This later insight was gained by using wild-caught *A. mexicanum* in the interspecific crossing design described above (Voss and Shaffer, 2000). Thus, continuous and discrete forms of metamorphic variation are explained by the same genetic factor and there is no need to invoke separate genetic mechanisms for the origin of discrete versus continuous phenotypic variation (Voss and Smith, 2005).

While it is clear that salamander paedomorphosis is associated with genetic changes that delay developmental timing, it is not clear when and how these changes manifest during development. In a general sense, it is important to determine when and how evolution acts during ontogeny to alter development because these insights help conceptualize the potential for evolution at micro and macroevolutionary levels (Raff and Wray, 1989; Wagner and Altenberg, 1996). The idea that paedomorphosis arises from few physiological or genetic changes suggests that ontogeny can be altered by modifying developmental switches, in this case acting somewhat discretely at the terminus of the larval period. Such a mechanism would allow regulation of metamorphic timing without pleiotropic modification of earlier growth and development. This type of regulatory control mechanism may be characteristic of modular developmental architectures that are evolutionarily labile (Gould, 1981). There is support for this idea; ambystomatid larvae that inherit the metamorphic *met* genotype develop at the same rate as larvae that inherit the paedomorphic *met* genotype (Voss, 1994). This pattern suggests that paedomorphic and metamorphic larvae are developmentally equivalent until late in the larval period, when brain regions (e.g. hypothalamus and pituitary) become competent to stimulate the release of TH and other hormones.

Alternatively, it is possible that variation in neuroendocrine function at the time of metamorphosis traces to differences in the way the brain matures during embryogenesis or early larval development. In considering how discontinuous phenotypes evolve via Darwinian means, Gould (1977b) proposed an answer in the case of the axolotl:

“the problem of reconciling evident discontinuity in macroevolution with Darwinism is largely solved by the observation that small changes early in embryology accumulate through growth to yield profound differences among adults.... Delay the onset of metamorphosis and the axolotl of Lake Xochimilco reproduces as a tadpole with gills and never transforms into a salamander.”

Thus, Gould proposed that discontinuous phenotypes arise from mutations that function during early development. Although once a controversial idea, it is generally accepted now that evolution can act on early stages of development to yield novel phenotypes

(Raff, 1996). What is important to note in the axolotl example is that there is a lengthy disconnect between the time a tiger salamander hatches and the time it first shows morphological changes indicative of metamorphosis (~ 100 days). Thus, there is considerable time for early ontogenetic events to alter brain development in a way that influences the release of TH late in the larval period. However, whether paedomorphic and metamorphic larvae show similar or different patterns of neurological development has not been investigated.

In this study, I examined the brains of larval *A. mexicanum* and *A. t. tigrinum* to test the hypothesis that paedomorphic and metamorphic larvae are developmentally equivalent until late in the larval period, when metamorphosis is initiated. A custom microarray was used to estimate mRNA abundances for thousands of genes at 42, 56, 70, and 84 days post hatching (dph). I identified genes that showed significant changes in gene expression across time within each species, and then compared the gene lists. Under the developmental equivalence hypothesis, I expected to identify the same differentially expressed genes (DEGs) between the species. In support of this hypothesis, 114 DEGs were identified in common between the species and all but 2 showed the same temporal pattern of expression. However, many DEGs were identified uniquely from each species. In particular, several genes that are associated with the hypothalamus-pituitary-interrenal axis, which is implicated in the regulation of amphibian metamorphosis, exhibited significant expression differences between *A. mexicanum* and *A. t. tigrinum* before 56 dph. The results below show that metamorphic and paedomorphic modes of development are associated with different transcriptional programs in the brain, and these programs diverge during early larval development.

CHAPTER TWO: METHODS AND MATERIALS

Study Animals

A single fertilized egg clutch of *A. t. tigrinum* was obtained in January of 2007 from Charles D. Sullivan Co. Inc. Upon hatching salamanders were reared individually according to established protocols (Voss, 1995; Voss et al., 2000; Voss and Smith, 2005). The following year *A. mexicanum* were obtained from a single inbred genetic cross and reared individually following hatching. Larvae from both species were reared in modified Holtfretter's solution (NaCl: 2.12M, NaHCO₃: 0.85M, KCl: 0.284M, CaCl₂: 0.284M, MgSO₄: 0.284M) at 20-22 C and fed brine shrimp napuli (*Artemia* sp., Brine Shrimp Direct, Ogden, UT) twice daily for three weeks. After three weeks post hatching, larvae were fed California blackworms (*Lumbriculus* sp., J.F. Enterprises, Oakdale, CA). At 28, 42, 56, 70, 84, and 98 dph, salamanders were anesthetized in 0.01% benzocaine (Sigma) and whole brain was collected (Fig. 2.1). Two different investigators collected the tissue samples from *A. mexicanum* and *A. t. tigrinum*. All *A. t. tigrinum* tissues were collected by Jeramiah Smith and I collected all of the *A. mexicanum* tissues. For all salamanders, mass, snout-vent length (SVL), and the number of front and hind toes were measured at each time point sampled. All procedures were approved by the University of Kentucky Animal Care and Use Committee (IACUC protocol # 01087L2006 and #00907L2005).

RNA Isolation

Total RNA was isolated from whole brain corresponding to three replicate pools of three individuals for each time point using TRIzol (Invitrogen, Carlsbad, CA). RNA samples were further purified using Qiagen RNeasy mini-columns. RNA samples were quantified via UV spectrophotometry (NanoDrop, ND-1000) and qualified via an Agilent BioAnalyzer (Agilent Technologies).

Gene Expression Profiling

Genome-level gene expression profiling was conducted using the custom Affymetrix GeneChip described in Page et al. (2007). Three RNA pools from each of four time points (42, 56, 70, 84 dph) were labeled and hybridized to an independent GeneChip and scanned by the University of Kentucky Microarray Core Facility according to standard Affymetrix protocols. Additional gene expression profiling for selected genes was conducted across all time points using quantitative reverse

transcriptase real-time polymerase chain reaction (qPCR). Primers (Table 2.1) were designed using Primer3 (Rozen and Skaletsky, 2000). *A. mexicanum* and *A. t. tigrinum* orthologs for each gene in Table 2.1 were aligned (using Basic Local Alignment Search Tool (BLAST)) to identify gene regions that corresponded to the same residues covered by Affymetrix probe-sets. When the orthologs were not 100% identical, separate primers were designed for each species to the target areas. Primer efficiency was estimated separately for *A. mexicanum* and *A. t. tigrinum* via linear regression on a dilution series. A BioRad iScript cDNA synthesis kit (Hercules, CA, USA) was used to synthesize cDNA from 1µg of RNA. A control gene (*Tif1*; probe-set L_s_at; Table 2.1; Page et al., 2008) was demonstrated to be invariant across all species by time combinations and relative expression ratios were calculated according to Pfaffl (2001). All expression ratios are relative to the average 28 dph expression values for *A. mexicanum* and normalized to *Tif1* (see above). All PCRs were 10µl reactions consisting of 5ng cDNA, 16.4ng of forward and reverse primers, and iQ SYBR-Green real-time PCR mix (Roche Diagnostics, Indianapolis, IN). PCRs were conducted on an Applied Biosystems StepOnePlus real-time PCR system. Cycle conditions were as follows: 10 minutes at 95°C, 40 cycles of 15 seconds at 95°C followed by 1 minute at 55°C, 15 seconds at 95°C, and 1 minute at 55°C. Melting curves were generated to ensure amplification of a single product for each reaction. All reactions were run on 48 well plates and blocked by sampling time and species (i.e. for a given time point, both species were present on the plate). At least two template free controls were present on each plate (Bustin and Nolan, 2004).

Quality Control and Low-Level Analyses of the Ambystoma GeneChip

All arrays were subjected to quality control (QC) at the individual probe level by inspecting box-plots, histograms, pair-wise M versus A plots of replicate GeneChips, images of the log₂ (intensity) values for each GeneChip, and an RNA degradation plot that allows for visualization of the 3' labeling bias across all GeneChips simultaneously (see Bolstad et al., 2005a, 2005b). Background correction, normalization, and expression summaries were achieved via the robust multi-array average (RMA) algorithm of Irizarry et al. (2003). RMA values were generated separately for each species. Upon implementing the RMA algorithm, the probe-set level data were subjected to further QC

by inspecting pair-wise M vs. A plots of replicate GeneChips, examining correlation matrices among replicate GeneChips (*A. mexicanum* min = 0.989357, *A. mexicanum* max = 0.9983847, *A. t. tigrinum* min = 0.9959386, *A. t. tigrinum* max = 0.9974595)

Probe-set Filtering

Because microarray experiments suffer from considerable multiple testing burdens, I filtered probe-sets that were classified as “present” on < 50% of the GeneChips in my experiment (McClintick and Edenber, 2006). However, because it is possible that some genes are expressed in one of the two species examined but not both, we retained probe-sets that were scored as “absent or marginal” in all of the GeneChips associated with one species but were scored as “present” in $\geq 50\%$ of the GeneChips associated with the other species. In some cases this resulted in the retention of probe-sets that were “present” on < 50% of the total GeneChips used in the experiment. These filters resulted in a total of 4133 probe-sets in the Mexican axolotl and 3963 probe-sets in the eastern tiger salamander that were available for significance testing.

Identification of Differentially Expressed Genes and Data Filtration: Microarray

Each species was analyzed separately using a step down quadratic regression approach (Liu et al., 2005) to identify differentially expressed genes (DEGs). This approach treats time as a continuous variable and allows for the identification of DEGs as a function of time. Additionally, this approach allows for the classification of DEGs based on their regression coefficients. This approach uses two levels of significance, α_0 and α_1 , where α_0 is used to identify DEGs, and α_1 is used to classify the DEGs into different regression patterns. The DEGs are classified into nine categories (Fig. 2.2); FLAT, linear up (LU), linear down (LD), quadratic concave (QC), quadratic convex (QV), quadratic linear concave up (QLCU), quadratic linear concave down (QLCD), quadratic linear convex up (QLVU), and quadratic linear convex down (QLVD). These expression profiles allow biologically meaningful temporal expression profiles to be obtained (Liu et al., 2005).

To correct for multiple testing I applied the false discovery rate (FDR) procedure of Benjamini and Hochberg (1995; FDR = 0.05) to the P-values associated with the overall model F-statistics. Upon performing FDR correction, a total of 1415 probe-sets in *A. mexicanum* and 1864 probe-sets in *A. t. tigrinum* were identified as differentially

expressed. An additional filter was then imposed in order to identify a smaller subset of probe-sets. I required that each probe-set exhibit a ≥ 1.5 -fold change at one or more of the four time points investigated to be a DEG. This yielded a total of 217 (15%) probe-sets from *A. mexicanum* and 465 (25%) probe-sets from *A. t. tigrinum*. To arrive at the final gene list, the probe-sets exhibiting the same expression profile and corresponding to the same human ortholog were combined and the probe-set exhibiting the largest fold-change was used. If probe-sets corresponding to the same human ortholog exhibited a different expression profile, these genes received a suffix identifier (i.e., different probe-sets for *hba2* had three different expression profiles, therefore they are listed as *HBA2_1*, *HBA2_2*, and *HBA2_3*) (Appendix I; Appendix II).

Identification of Gene Expression Patterns

To complement the regression modeling approach, DEGs were classified into temporal patterns according to the method of Monaghan et al. (2007). This approach assigns gene expression scores at each time point that mRNA abundances are estimated. The earliest sample time (i.e. 42 dph) is considered the base line mRNA abundance. If the mRNA abundance for a gene is < 1.5 -fold deviant from baseline, the gene receives a score of non-significant (N) at the next sample time. If the mRNA abundance deviates from baseline by ≥ 1.5 -fold, the gene receives a score of up regulated (U) or down regulated (D). Thus, for the 56 dph sample time a gene could receive a score of U, D, or N. For the 70 and 84 dph sample times, a gene could either be N, U, D, or maintain constant (C) mRNA abundance. If a gene was < 1.5 -fold deviant from the previous sample time, then the gene received a score of C. A score of C was never assigned after a score of N. Under this scoring scheme, a gene received either N, U, or D score at 56 dph and either a N, U, D, or C score at 70 and 84 dph sample times. A complete breakdown of the significant genes and their gene expression pattern is shown in Appendix I for *A. mexicanum* and Appendix II for *A. t. tigrinum*. More than half of the DEGs (N = 113 for *A. mexicanum*, N = 272 for *A. t. tigrinum*) showed amino acid sequence identity (BLASTx, $e < 1 \times 10^{-7}$) to a presumptive human protein-coding gene. I assumed that these sequence matches established orthology and further assumed that salamander-human orthologs function in the same biological process. This allowed me to annotate human gene functions to DEGs. Annotation information was obtained via multiple databases

(GO, OMIM, KEGG, etc.). However, the annotations are biased in order to emphasize possible gene functions in brain development and metamorphosis

Statistical Analyses of the qPCR Data

I used polynomial regression to model the gene expression profiles for *A. mexicanum* and *A. t. tigrinum* that were generated via qPCR. A backward selection scheme was used to identify what order polynomial to accept as a final model. Thus I started with a full fifth-order model of the general form $\log_2(R)_{ijk} = \mu_0 + S_t + T + (ST)_t + T^2 + (ST^2)_t + T^3 + (ST^3)_t + T^4 + (ST^4)_t + T^5 + (ST^5)_t + \varepsilon_{ijk}$ where μ_0 is the intercept term that is added to all observations, S_t = a species specific term that is added only to observations of *A. t. tigrinum*, T = the time effect, $T^2 \dots T^5$ = the effects of various orders of time, and $(ST)_t \dots (ST^5)_t$ = the interaction between species and the various orders of time effect, and ε_{ijk} = the error term associated with individual k at time point j , from species i . I then implemented a multiple regression approach in which terms with $P > 0.05$ were removed from the model until all terms remaining in the model had corresponding P -values < 0.05 . This scheme was applied with the constraint that the main effects underlying significant interaction terms were retained irrespective of the significance level of these main effects.

TABLES AND FIGURES

Table 2.1: Primer sequences used for qPCR.

Gene	Symbol	Probe-Set ID	Designed to	Sequence	Direction
<i>Proopiomelanocortin</i>	<i>POMC</i>	SRV_10930_at	<i>A. t. tigrinum</i>	AGATGGCACTACAGGATGC	5.1
<i>Proopiomelanocortin</i>	<i>POMC</i>	SRV_10930_at	<i>A. t. tigrinum</i>	ACTCCGTTTCAGGGTTCAT	3.1
<i>Glutamate-ammonia ligase (Glutamine Synthetase)</i>	<i>GLUL</i>	SRV_11340_at	Both	ACTTTGGCGTTGTGGCTA	5.1
<i>Glutamate-ammonia ligase (Glutamine Synthetase)</i>	<i>GLUL</i>	SRV_11340_at	Both	TGGCTTCCGTGCTGTAGT	3.1
<i>Nuclear receptor subfamily 3, group C, member 2</i>	<i>NR3C2</i>	SRV_00540_a_at	<i>A. t. tigrinum</i>	TGGGAGCTCGAAAGTCAA	5.1
<i>Nuclear receptor subfamily 3, group C, member 2</i>	<i>NR3C2</i>	SRV_00540_a_at	<i>A. t. tigrinum</i>	AGGTGGTTGTGGCTGTTG	3.1
<i>Nuclear receptor subfamily 3, group C, member 2</i>	<i>NR3C2</i>	SRV_00540_a_at	<i>A. mexicanum</i>	TGGGAGCTCGAAAGTCAA	5.1
<i>Nuclear receptor subfamily 3, group C, member 2</i>	<i>NR3C2</i>	SRV_00540_a_at	<i>A. mexicanum</i>	AGGTGGTTGTGGCTGTTG	3.1
<i>Junonji AT rich interactive domain 2</i>	<i>JARID2</i>	SRV_02395_a_at	Both	TCGAAAGTGGCTGCAGTT	5.1
<i>Junonji AT rich interactive domain 2</i>	<i>JARID2</i>	SRV_02395_a_at	Both	GTGCTCCTGCACAACCAT	3.1
<i>Hemoglobin, Gamma A</i>	<i>HBG1</i>	SRV_00434_copy1_x_at	<i>A. mexicanum</i>	ACGCACGACGAGAAAAGA	5.1
<i>Hemoglobin, Gamma A</i>	<i>HBG1</i>	SRV_00434_copy1_x_at	<i>A. mexicanum</i>	TCTGGCGAGACTTTCAGC	3.1
<i>Hemoglobin, Gamma A</i>	<i>HBG1</i>	SRV_00434_copy1_x_at	<i>A. t. tigrinum</i>	GCGATCAAGTAGGAGCTGAA	5.1
<i>Hemoglobin, Gamma A</i>	<i>HBG1</i>	SRV_00434_copy1_x_at	<i>A. t. tigrinum</i>	GCCAAATTGCGAAATTTCT	3.1
<i>Hemoglobin, Epsilon 1</i>	<i>HBE1</i>	SRV_00186_a_at	<i>A. mexicanum</i>	AGGTCTTGTGGCTCTGG	5.1
<i>Hemoglobin, Epsilon 1</i>	<i>HBE1</i>	SRV_00186_a_at	<i>A. mexicanum</i>	GCGTGTGAGCATGAATCTC	3.1
<i>Hemoglobin, Epsilon 1</i>	<i>HBE1</i>	SRV_00186_a_at	<i>A. t. tigrinum</i>	TTGGCCAAATCGAGTGAGA	5.1
<i>Hemoglobin, Epsilon 1</i>	<i>HBE1</i>	SRV_00186_a_at	<i>A. t. tigrinum</i>	AAGACGATCACCAGGCAGT	3.1
<i>Hemoglobin, Delta</i>	<i>HBD</i>	SRV_00374_at	<i>A. mexicanum</i>	CCCCGAATTGGAAGACG	5.1
<i>Hemoglobin, Delta</i>	<i>HBD</i>	SRV_00374_at	<i>A. mexicanum</i>	TGCGTAAATCGCACGAC	3.1
<i>Hemoglobin, Delta</i>	<i>HBD</i>	SRV_00374_at	<i>A. t. tigrinum</i>	CACCTGACAGCCGAAGAA	5.1
<i>Hemoglobin, Delta</i>	<i>HBD</i>	SRV_00374_at	<i>A. t. tigrinum</i>	TTGCAAGGCATTGACCTC	3.1
<i>Hemoglobin, Alpha 1</i>	<i>HBA2</i>	SRV_02509_copy4_at	<i>A. mexicanum</i>	GCACGCCTACAACCTGAG	5.1
<i>Hemoglobin, Alpha 1</i>	<i>HBA2</i>	SRV_02509_copy4_at	<i>A. mexicanum</i>	GAAGTCAGCTGGGAAGTGG	3.1
<i>Hemoglobin, Alpha 1</i>	<i>HBA2</i>	SRV_02509_copy4_at	<i>A. t. tigrinum</i>	GCCTACAACCTGCGAGTG	5.1
<i>Hemoglobin, Alpha 1</i>	<i>HBA2</i>	SRV_02509_copy4_at	<i>A. t. tigrinum</i>	GGTGAAGTCAGCTGGGAAG	3.1
<i>Nuclear receptor subfamily 3, group C, member 1</i>	<i>NR3C1</i>	N/A	<i>A. mexicanum</i>	AGCGCACAGGTAGTTGTGTT	5.1
<i>Nuclear receptor subfamily 3, group C, member 1</i>	<i>NR3C1</i>	N/A	<i>A. mexicanum</i>	ACTACGGTGTCTTGACGTGT	3.1
<i>Tubulin, Beta 2C</i>	<i>TUBB2C</i>	AG_s_at	<i>A. mexicanum</i>	CGTTGGGTCGACCATTTA	5.1
<i>Tubulin, Beta 2C</i>	<i>TUBB2C</i>	AG_s_at	<i>A. mexicanum</i>	AAGCCGACATCCCTGAAC	3.1
<i>Tubulin, Beta 2C</i>	<i>TUBB2C</i>	AG_s_at	<i>A. t. tigrinum</i>	TACGGCGATCTCAACCAC	5.1
<i>Tubulin, Beta 2C</i>	<i>TUBB2C</i>	AG_s_at	<i>A. t. tigrinum</i>	CGGAGATCTGCATTGAGC	3.1
<i>Corticotropin releasing hormone receptor 1</i>	<i>CRHR1</i>	N/A	Both	TCTGAGATGGGGCAAAGA	5.1
<i>Corticotropin releasing hormone receptor 1</i>	<i>CRHR1</i>	N/A	Both	TCATTGCCATGCCATTCT	3.1
<i>Retinoic acid induced 1</i>	<i>RAI1</i>	N/A	<i>A. mexicanum</i>	CAATCCGCAGTCTTTCCA	5.1
<i>Retinoic acid induced 1</i>	<i>RAI1</i>	N/A	<i>A. mexicanum</i>	CCTTGTTTGGAGCTGCTG	3.1
<i>Dexamethasone-induced 1</i>	<i>RASD1</i>	N/A	<i>A. mexicanum</i>	AGCAGGCACACATTGCTT	5.1
<i>Dexamethasone-induced 1</i>	<i>RASD1</i>	N/A	<i>A. mexicanum</i>	GAGCCCAGGATGACCATT	3.1
<i>Transcriptional intermediary factor 1</i>	<i>TIF1</i>	L_s_at	<i>A. mexicanum</i>	CCTCTGGTCTCGGATCG	5.1
<i>Transcriptional intermediary factor 1</i>	<i>TIF1</i>	L_s_at	<i>A. mexicanum</i>	TCCTGCGGAGGCTGTT	3.1

Probe-set ID represents the unique identifier for each probe-set on the custom *Ambystoma* GeneChip. "Both" represents primers with no sequence divergence and therefore designed from *A. mexicanum* and *A. t. tigrinum* sequence. Forward and reverse primers are denoted by 5.1 and 3.1 respectively.

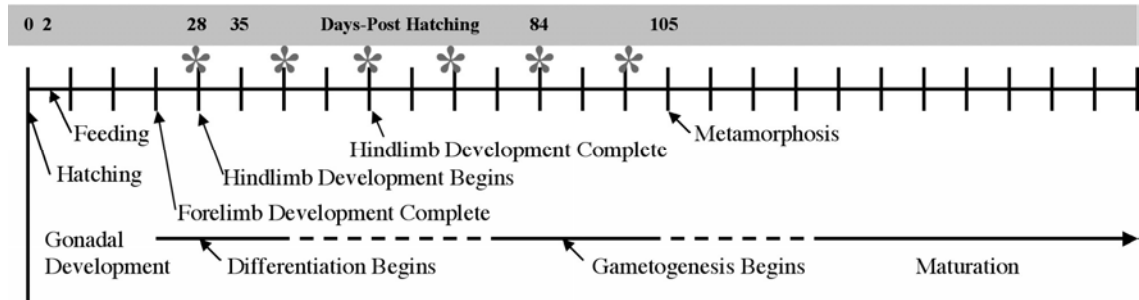


Figure 2.1: Timing of developmental events leading up to metamorphosis in *A. mexicanum* and *A. t. tigrinum*. Asterisks correspond to time points used in microarray and qPCR.

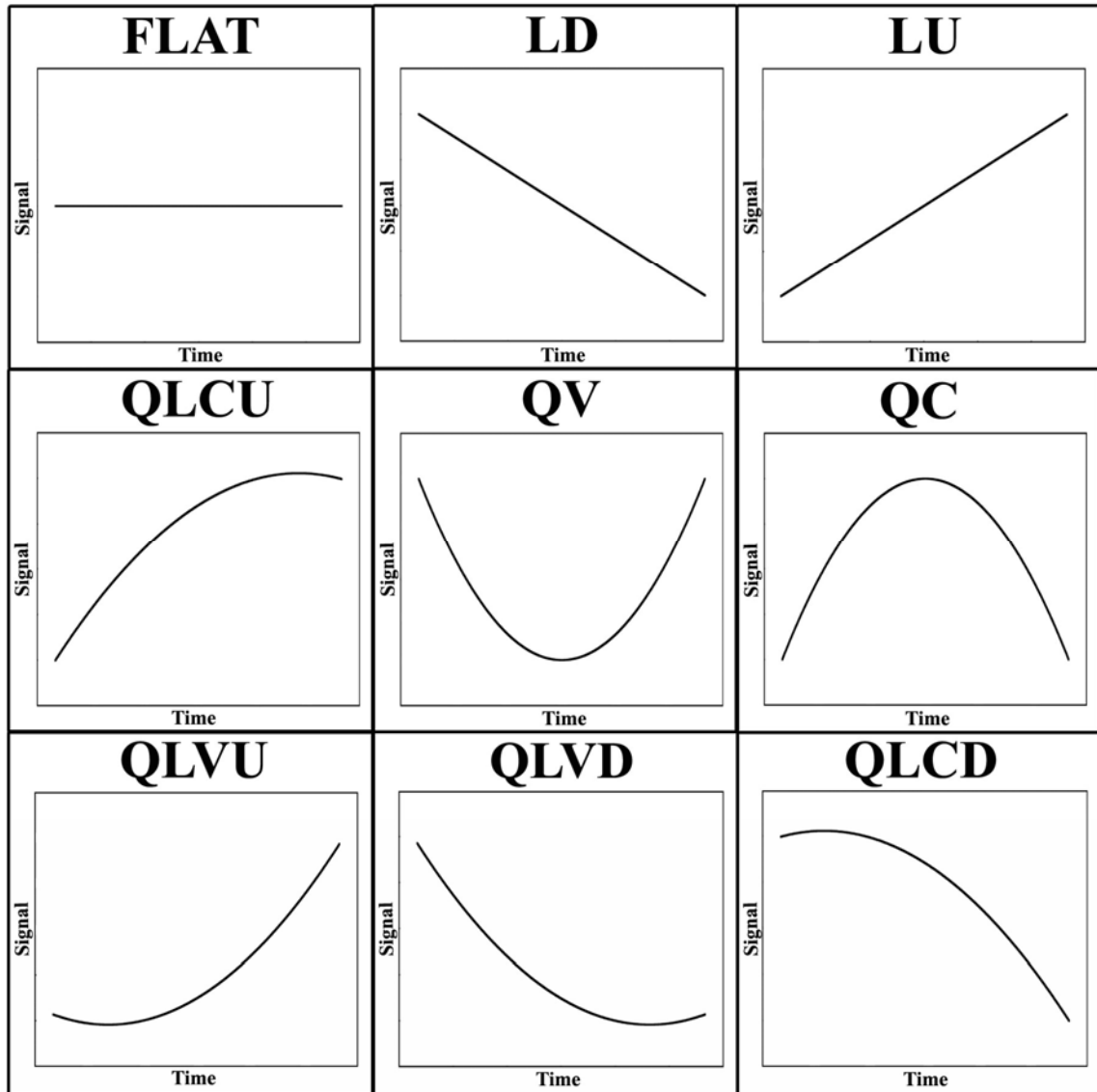


Figure 2.2: Nine expression profiles identified by the Lui et al. (2005) methodology. While the units and values of the axes are arbitrary, the y-axis represents the hybridization signals obtained from the microarray analysis and the x-axis represents the log transformation of time. FLAT denotes no significant difference over time; LD denotes linear down regulated patterns; LU denotes linear up regulated patterns; QLCU denotes quadratic linear concave up regulated patterns; QV denotes quadratic convex regulated patterns; QC denotes quadratic concave regulated patterns; QLVU denotes quadratic linear convex up regulated patterns; QLVD denotes quadratic linear convex down regulated patterns; QLCD denotes quadratic linear concave down regulated patterns.

CHAPTER THREE: RESULTS

Growth and Differentiation Rate

The *A. t. tigrinum* and *A. mexicanum* larvae that were used in this study were reared under the same husbandry conditions but not at the same time. To assess whether this contributed to variation in development beyond that expected between these two species, I compared the change in overall growth and the timing of toe differentiation. It is known that *A. t. tigrinum* lays larger eggs than *A. mexicanum* and thus larvae hatch at larger body sizes (Voss, personal comm.). This pattern was also observed for the larvae used in my experiment. The *A. t. tigrinum* maintained a larger body size throughout the larval period (Fig. 3.1). With respect to developmental rate, the same number of forelimb toes was observed between *A. t. tigrinum* and *A. mexicanum* larvae at 28 dph. At 42 dph, there was variation in the number of hindtoes between the species, with *A. t. tigrinum* larvae showing a median of 4 toes and *A. mexicanum* larvae showing a median of 3 toes. This suggests that *A. t. tigrinum* were more developmentally advanced at this time, however this difference is slight because by 56 dph, all larvae of both species had completed hindlimb development. These results suggest that the larvae used in this experiment were sampled at approximately equivalent stages of development.

Differentially Expressed Genes

Statistical and fold-level criteria were used to identify DEGs for each species. More genes were differentially expressed during larval development in *A. t. tigrinum* (N=395) than in *A. mexicanum* (N=185) (Appendix II; Appendix I). There was considerable overlap between the species as more than half of the DEGs (N=113) identified from *A. mexicanum* were also identified from *A. t. tigrinum* (Fig. 3.2). The remaining DEGs identified from each species were uniquely differentially expressed. Thus, many shared and unique DEGs were identified, with more unique DEGs identified for *A. t. tigrinum*.

Gene Expression Patterns

The DEGs identified from each species were classified into gene expression categories using regression modeling (Liu et al., 2005) and the approach of Monahan et al. (2007). Of nine possible regression models (Fig. 1.2), six were observed in *A. mexicanum* (LU, LD, QLCU, QLCD, QLVU, and QLVD) and seven were observed in *A.*

t. tigrinum (LU, LD, QC, QLCU, QLCD, QLVU, and QLVD). The majority of the shared and unique DEGs (65%) fit linear regression models (LU and LD) in both species. This suggests that many genes showed a linear increase or decrease in expression between 42 and 84 dph. Although only 40% of the shared DEGs were classified into the same regression model, the change in mRNA abundance was always in the same direction. For example, *mlf1* was classified as LU in *A. t. tigrinum* but QLVU in *A. mexicanum*. Thus, although there were subtle differences in the shape of regression profiles for genes that were differentially expressed in both *A. mexicanum* and *A. t. tigrinum*, the patterns of change were in the same direction.

The approach of Monaghan et al. (2007) was then used to identify the sample times that DEGs showed significant fold-level changes (≥ 1.5 fold). Although 47 gene expression patterns were possible under this method, only 11 and 16 different gene expression patterns were observed for *A. mexicanum* and *A. t. tigrinum* respectively (Appendix I; Appendix II). Of the patterns observed, only a small percentage (~10%) of shared and unique DEGs exhibited significant fold change in mRNA abundance between 42 and 56 dph. However, between 56 and 70 dph, 54% of the shared DEGs and 27% (*A. mexicanum*) and 32% (*A. t. tigrinum*) of the unique DEGs showed a significant fold change. Then, between 70 and 84 dph, ~32% of the shared DEGs and 64% (*A. mexicanum*) and 61% (*A. t. tigrinum*) of the unique DEGs showed a significant fold change. Thus, shared DEGs were more likely to show a 1.5 fold change between 56 and 70 dph, while unique DEGs were more likely to show a 1.5 fold change at the latest time point. These patterns suggest the following: 1) Gene expression is more similar between *A. mexicanum* and *A. t. tigrinum* at early versus later stages of larval development, 2) As larval development proceeds, more genes are expressed differently between *A. mexicanum* and *A. t. tigrinum*.

Presumptive Biological Functions of Shared and Unique DEGs

The DEGs that showed sequence identity to a human RefSeq protein were assumed to be salamander-human orthologs. These DEGs were annotated with functional information from the Gene Ontology database, the Gene Database at NCBI, and articles from PubMed. As might be expected when profiling temporal gene expression for a complex organ like the brain, DEGs were associated with many different biological

processes. Many of the same biological process categories were represented among the three DEG lists – the DEGs that were expressed in common between the species (i.e. shared list) (Table 3.1) and the separate lists of DEGs that were identified uniquely from *A. mexicanum* (Table 3.2) and *A. t. tigrinum* (Table 3.3). The shared list yielded 24 different biological process categories. The cell cycle category was supported by the most genes (N=22), followed by immune/stress response (N=9), transcription (N=7), and oxygen transport (N=5). All but two of the cell cycle genes and all but 1 of the transcription genes showed a pattern of down regulation in both species. For example, *sox3* and *msx1*, two classical markers of neural development and differentiation (e.g. Freed et al., 2008; Nikcevic et al., 2008), showed 1.5 fold decreases in expression between 56 and 70 dph. Also at this time, all but two of the cell cycle genes showed a 1.5 fold decrease in expression. These patterns suggest that some aspects of neural development are similarly regulated between *A. t. tigrinum* and *A. mexicanum* during larval development. This also seems to be the case for non-neuronal cells that were also likely sampled from the brain, including stress/immune response and oxygen transport DEGs that were presumably isolated from red blood cells and lymphocytes.

Most, but not all of the biological process categories that were observed within the shared DEG list were also observed in the *A. mexicanum* unique list (Table 3.2). Moreover, the distribution of genes was different for categories that were observed in both lists. For example, fewer genes in the *A. mexicanum* unique list were annotated as functioning in the cell cycle (N = 2) and no transcription factors were identified as DEGs. The most highly represented category was apoptosis (N=5), a category that was not observed among the shared gene list. Overall, relatively few genes supported each functional category. However, DEGs that are implicated in brain development, including genes that code for extracellular matrix constituents (*dpt*, *coll1a1*), and genes that function in cell adhesion (*coll1a1*, *dpt*, *lgals4*, *dcn*), and apoptosis (*hebp2*, *ctss*, *mtch1*, *capns1*) were uniquely up regulated in *A. mexicanum*. Also, several biomarkers of mammalian brain pathologies were uniquely up regulated in *A. mexicanum* (*ctss*, *cd69*, *ogn*, and *dcn*). These and other uniquely expressed genes from *A. mexicanum* may similar serve as candidate biomarkers for the paedomorphic mode of development.

The larger list of unique DEGs from *A. t. tigrinum* yielded more biological process annotations (Table 3.3). As was observed for the shared gene list, the most abundant functional category was cell cycle (N=26), and the transcription (N=14) category was also highly represented. Also similar to the results from the shared gene list, almost all of the cell cycle and transcription genes showed decreasing mRNA abundances during larval development. For example, *lmx1b* showed a pattern of decreasing transcript abundance, as did several other genes that function in chromatin modification and gene silencing (e.g. *dnmt1*, *baz1b*, *baz1a*, *smarca5*). These epigenetic functional categories were not observed in the shared gene list or the unique *A. mexicanum* list. The general pattern of down-regulated expression was also observed for genes from the DNA replication (N=9) and RNA binding categories (N=10), and more generally, for genes that are predicted to have nuclear-localized functions (DNA binding, nuclear transport, response to DNA damage, and RNA splicing and processing). To reiterate a point made earlier, the majority of these unique *A. t. tigrinum* DEGs showed significant fold-level changes late in the larval period.

Several additional functional categories were observed between the shared and unique *A. t. tigrinum* DEG lists, including stress/immune response, cell differentiation/maturation, and signal transduction. Whereas the stress/immune response genes showed increasing abundances in the shared DEG list (N=9), both up (N=6) and down-regulated (N=8) patterns were observed in the unique *A. t. tigrinum* list. There were also more unique *A. t. tigrinum* DEGs annotated for the protein binding (N=11) and signal transduction (N=10) categories, and the majority of these were up regulated between 70 and 84 dph. It is possible that some of these and other unique *A. t. tigrinum* DEGs are associated with the development and function of brain regions that orchestrate later metamorphic events. For example, several genes that function to regulate the secretion of hypothalamic, pituitary, and interrenal hormones were uniquely expressed in *A. t. tigrinum*, including *mineralocorticoid receptor (nr3c2)*, *somatostatin receptor 5 (sstr5)*, *mediator complex subunit 24 (med24)*, *scaffold attachment factor b (scafb)*, *nuclear receptor coactivator 5 (ncoa5)*. Also, *prolactin (prl)*, which is known to increase in abundance during anuran metamorphosis (Buckbinder and Brown, 1993; Takahashi et al., 1990), only increased significantly in *A. t. tigrinum*. In addition to these genes,

proopiomelanocortin (pomc) and *corticotrophin releasing hormone receptor (crhr1)*, exhibited higher expression levels during larval development in *A. t. tigrinum*. Thus, the microarray analysis identified several genes whose differential expression between *A. t. tigrinum* and *A. mexicanum* maybe associated with metamorphic and paedomorphic modes of development.

Further Investigation of Vertebrate Neuroendocrine Axis Genes using qPCR

Some of the DEGs that were identified by microarray analysis suggest that the hypothalamus-pituitary-adrenal (interrenal in amphibians) axis is differentially regulated between *A. mexicanum* and *A. t. tigrinum*. To investigate this idea further, we used qPCR to examine the expression of *nr3c2*, *pomc*, *glucocorticoid receptor (nr3c1)*, and *crhr1* at the same time points that were examined in the microarray analysis, plus two additional time points (28 and 98 dph). Similar temporal patterns were observed between *A. mexicanum* and *A. t. tigrinum* for *pomc*, *nr3c2*, and *crhr1*, however, transcript abundances were significantly higher in *A. t. tigrinum* (Fig. 3.3). The qPCR results validated the microarray results by showing a significant difference in *nr3c2* transcript abundances between *A. t. tigrinum* and *A. mexicanum* before 56 dph. Indeed, the expression profiles appear to diverge as early as 42 dph. Also, qPCR validated the invariant expression of *nr3c1* between the species. Finally, qPCR validated the small but significant mRNA abundance estimates obtained by microarray analysis for *pomc* and *crhr1*. If mRNA abundance for these genes correlates with HPI activity, these results suggest that the HPI axis is more active in the metamorphic *A. t. tigrinum* than in the paedomorphic *A. mexicanum*.

Biological Replication and Verification

To further validate the microarray results, a sample of hemoglobin and metabolic genes were also investigated using qPCR. Tables 3.4 and 3.5 show the results of the microarray analysis alongside the results of the qPCR regression analysis. I failed to replicate only the magnitude of the fold change estimated via microarray analysis and qPCR for some of the genes. However, the genes that failed verification registered a low-fold change and were not significant in one of the species in the microarray analysis. While the magnitude of the fold change differed for some of the genes, the directional trends were validated. Overall, the qPCR results validated the microarray results.

TABLES AND FIGURES

Table 3.1: DEGs expressed in *A. mexicanum* and *A. t. tigrinum* by ≥ 1.5 -fold.

Gene Symbol	Mex Pattern	Mex Model	Tig Pattern	Tig Model	Gene Function
RPL31	DCC	QLVD	NND	LD	Protein synthesis
Unknown	DCC	QLVD	NDC	QLVD	Unknown
Unknown	DDC	QLVD	DCC	QLVD	Unknown
UHRF1	NDC	QLVD	DDD	LD	Cell cycle
ILF3	NDC	QLVD	NDC	LD	Cell cycle
CCNA2	NDC	QLVD	NDD	QLCD	Cell cycle: Checkpoint
UHRF2	NDC	LD	NDD	LD	Cell cycle: Regulation
CHEK1	NDC	LD	NDC	LD	Cell cycle; DNA damage
RRM2	NDC	QLVD	DDD	LD	Cell cycle; DNA replication
RRM1	NDC	LD	NDD	QLCD	Cell cycle; DNA replication
MCM7	NDC	QLVD	NDD	LD	Cell cycle; DNA replication
MCM3	NDC	QLVD	NDD	QLCD	Cell cycle; DNA replication
RCC1	NDC	LD	DCC	QLVD	Cell cycle; M
PLK1	NDC	LD	NDD	QLCD	Cell cycle; M
MAD2L1	NDC	QLVD	NDD	LD	Cell cycle; M
KIFC1	NDC	QLVD	NDD	LD	Cell cycle; M
CDC20	NDC	QLVD	NDD	QLCD	Cell cycle; M
RCC2	NDC	LD	NND	LD	Cell cycle; M
KIAA0101	NDC	LD	NDD	QLCD	Cell cycle; S
H3F3B	NDC	LD	NDC	LD	DNA metabolism
MCM4	NDC	LD	NDD	QLCD	DNA metabolism
LAMA1	NDC	LD	NDC	LD	ECM component
PTBP1	NDC	LD	NDC	LD	Metabolism
PFAS	NDC	LD	NDC	LD	Metabolism
SLBP	NDC	QLVD	NDC	LD	mRNA processing
COL2A1	NDC	LD	DCC	QLVD	Organ development
TEAD4	NDC	LD	NDC	LD	Transcription
SPEN	NDC	QLVD	NDC	QLVD	Transcription
MSX1	NDC	LD	NDC	LD	Transcription
SOX3	NDC	LD	NDC	LD	Transcription factor
Unknown	NDC	LD	NDC	LD	Unknown
Unknown	NDC	LD	NDC	LD	Unknown
Unknown	NDC	LD	NDC	LD	Unknown
Unknown	NDC	QLVD	NDC	LD	Unknown
Unknown	NDC	QLVD	NDC	LD	Unknown
Unknown	NDC	LD	NDD	QLCD	Unknown
Unknown	NDC	LD	NDD	QLCD	Unknown
Unknown	NDC	LD	NND	LD	Unknown
Unknown	NDC	LD	NND	LD	Unknown
HBZ	NDD	QLCD	DDD	LD	Oxygen transport
CDC6	NND	LD	NDC	LD	Cell cycle; DNA replication
CDC25A	NND	LD	NDC	LD	Cell cycle; G1/S

Table 3.1 (Continued)

C6ORF115	NND	QLVD	NDC	LD	Cell cycle; M
MCM6	NND	QLVD	NDD	QLCD	Cell cycle; S
CHD4	NND	LD	NDC	LD	DNA metabolism
RGPD2	NND	LD	NDC	LD	Intracellular transport
TMPO	NND	LD	NDC	LD	Transcription
KHSRP	NND	LD	NDC	QLVD	Transcription
Unknown	NND	LD	NDC	LD	Unknown
Unknown	NND	LD	NDC	LD	Unknown
Unknown	NND	QLVD	NDC	LD	Unknown
Unknown	NND	LD	NDD	QLCD	Unknown
Unknown	NND	QLVD	NDD	LD	Unknown
Unknown	NND	LD	NND	LD	Unknown
Unknown	NND	LD	NND	LD	Unknown
Unknown	NND	LD	NND	LD	Unknown
Unknown	NND	LD	NND	LD	Unknown
Unknown	NND	LD	NND	LD	Unknown
Unknown	NND	LD	NND	LD	Unknown
Unknown	NND	LD	NND	QLCD	Unknown
SEPT8	NNU	QLCU	NUC	LU	Cell cycle
MLF1	NNU	QLVU	NNU	LU	Cell differentiation
ANG	NNU	LU	NNU	LU	Cell differentiation
ANXA1	NNU	LU	UCC	QLCU	Cell differentiation
FXYD3	NNU	LU	NNU	LU	Ion transport
ATP6V1C1	NNU	LU	NNU	LU	Metabolism
CNDP1	NNU	LU	NNU	LU	Peptidase activity
C10ORF54	NNU	LU	NNU	QLVU	Receptor activity
NPY	NNU	LU	NNU	LU	Stimulus response
TYROBP	NNU	LU	NNU	LU	Stress/Immune response
B2M	NNU	LU	NUU	LU	Stress/Immune response
Unknown	NNU	LU	NNU	LU	Unknown
Unknown	NNU	LU	NNU	LU	Unknown
Unknown	NNU	LU	NNU	LU	Unknown
Unknown	NNU	LU	NNU	LU	Unknown
Unknown	NNU	LU	NUU	LU	Unknown
Unknown	NNU	LU	NUU	QLVU	Unknown
Unknown	NNU	LU	UCU	LU	Unknown
LOH11CR2A	NUC	QLCU	NNU	LU	Cell cycle
LGALS1	NUC	LU	NUC	LU	Cell differentiation
ANXA1_2	NUC	LU	NUU	LU	Cell differentiation
CLU	NUC	LU	UCC	LU	Cell differentiation
CDH1	NUC	QLCU	NNU	LU	Cell-cell adhesion
FGFBP1	NUC	LU	UCC	QLCU	FGF signaling
ITGB1BP3	NUC	QLCU	NNU	QLVU	Metabolism
TMEM176B	NUC	QLCU	NUC	LU	Organ morphogenesis

Table 3.1 (Continued)

HBA2_1	NUC	QLCU	NDD	QLCD	Oxygen transport
CD9	NUC	LU	NUC	LU	Signal transduction
CD74	NUC	LU	NNU	LU	Stress/Immune response
IGLL1	NUC	LU	NUU	LU	Stress/Immune response
APOE	NUC	LU	NUC	LU	Stress/Immune response
SERPINH1	NUC	LU	UDC	QC	Stress/Immune response
CST3	NUC	LU	NUC	LU	Tissue remodeling
POLR2L	NUC	LU	NUU	QLVU	Transcription
Unknown	NUC	LU	NNU	LU	Unknown
Unknown	NUC	LU	NNU	LU	Unknown
ADH4	NUC	LU	NNU	LU	Unknown
Unknown	NUC	LU	NUC	LU	Unknown
Unknown	NUC	LU	NUC	LU	Unknown
Unknown	NUC	LU	NUC	LU	Unknown
Unknown	NUC	LU	NUC	LU	Unknown
Unknown	NUC	LU	UCC	QLCU	Unknown
PLA2G4C	NUU	LU	NUC	LU	Stress/Immune response
CNDP2	UCC	LU	NNU	LU	Dipeptidase activity
FAAH	UCC	QLCU	NNU	QLVU	Fatty acid hydrolysis
ALAS2	UCC	QLCU	NUC	LU	Stress/Immune response
CAT	UCC	LU	UCU	LU	Stress/Immune response
Unknown	UCC	LU	NNU	QLVU	Unknown
FTH1	UUC	QLCU	UUU	LU	Iron homeostasis
HBD	UUC	QLCU	UCC	QLCU	Oxygen transport
HBA2_2	UUC	QLCU	UCC	QLCU	Oxygen transport
HBG1	UUC	QLCU	UCC	QLCU	Oxygen transport

The column “Mex Pattern” describes the gene expression patterns on days 56, 70, and 84 post hatching and the column “Mex Model” describes the regression models fit to the gene expression patterns on days 42, 56, 70, and 84 post hatching for *A. mexicanum*. The column “Tig Pattern” describes the gene expression patterns on days 56, 70, and 84 post hatching and the column “Tig Model” describes the regression models fit to the gene expression patterns on days 42, 56, 70, and 84 post hatching for *A. t. tigrinum*. All genes were expressed by ≥ 1.5 -fold in *A. mexicanum* and *A. t. tigrinum*.

Table 3.2: DEGs uniquely expressed in *A. mexicanum* by ≥ 1.5 -fold.

Gene Symbol	Pattern	Model	Gene Function
MMP1	DCC	LD	Tissue remodeling
Unknown	DCC	QLVD	Unknown
Unknown	DCC	QLVD	Unknown
Unknown	DCC	QLVD	Unknown
PIM1	NDC	LD	Negative regulation of apoptosis
PLK3	NDC	LD	Cell cycle: Regulation
EWSR1	NDC	QLVD	RNA binding
LMNB2	NDC	LD	Structural molecule activity
Unknown	NDC	LD	Unknown
Unknown	NDC	QLVD	Unknown
Unknown	NDC	LD	Unknown
FLNA	NND	LD	Actin binding
DDX5	NND	LD	ATP binding
AKAP8L	NND	LD	Ion/protein binding
SERBP1	NND	QLCD	mRNA binding
EIF4G1	NND	LD	mRNA processing
RABAC1	NND	QLCD	Protein binding
TMED10	NND	LD	Protein transport
CDK9	NND	LD	Cell cycle: Regulation
Unknown	NND	LD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	QLVD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	QLVD	Unknown
ACTG2	NNU	LU	Actin binding
MTCH1	NNU	LU	Apoptosis
CAPNS1	NNU	LU	Apoptosis
LGALS4	NNU	LU	Cell adhesion
IDUA	NNU	LU	Hydrolysis
DMBT1	NNU	LU	Immune system
PCTP	NNU	LU	Lipid transport
DGAT2	NNU	QLCU	Metabolism

Table 3.2 (Continued)

BHMT	NNU	LU	Metabolism
ALG13	NNU	QLCU	Metabolism
GSTP1	NNU	LU	Neuroprotection
KIAA0776	NNU	LU	Protein binding
PPIC	NNU	LU	Signal transduction
CD69	NNU	QLVU	Signal transduction
ANXA5	NNU	LU	Signal transduction
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	QLVU	Unknown
HEBP2	NUC	LU	Apoptosis
CTSS	NUC	QLCU	Apoptosis
KRT5	NUC	QLCU	Cytoskeleton
DNTT	NUC	LU	DNA replication
DPT	NUC	LU	ECM component
COL1A1	NUC	QLCU	ECM component
OGN	NUC	LU	Growth factor activity
DCN	NUC	QLCU	Organ morphogenesis
SERPINB10	NUC	QLCU	Protein binding
Unknown	NUC	LU	Unknown
Unknown	NUC	QLCU	Unknown
Unknown	NUC	LU	Unknown
LOC390940	NUU	LU	Unknown
Unknown	UCC	QLCU	Unknown
Unknown	UCC	QLCU	Unknown

The column "Pattern" describes the gene expression patterns on days 56, 70, and 84 post hatching and the column "Model" describes the regression models fit to the gene expression patterns on days 42, 56, 70, and 84 post hatching for genes uniquely expressed by ≥ 1.5 -fold in *A. mexicanum*.

Table 3.3: DEGs uniquely expressed in *A. t. tigrinum* by ≥ 1.5 -fold.

Gene Symbol	Pattern	Model	Gene Function
SHMT1	DCC	QLVD	Metabolism
PSAP	DCC	LD	Metabolism
HBA2_3	DCC	QLVD	Oxygen transport
Unknown	DCC	LD	Unknown
C17ORF67	DCC	QLVD	Unknown
Unknown	DCD	LD	Unknown
KRT12	DDC	QLVD	Cytoskeleton
S100A2	DDC	LD	Ion binding
PPARD	DDC	LD	Transcription/Steroid hormone receptor
JARID2	DUC	QLVU	Central nervous system development
Unknown	DUC	QLVU	Unknown
CASP2	NDC	LD	Apoptosis
MYL9	NDC	QLVD	Biological process
COL11A1	NDC	LD	Cell adhesion
PCNA	NDC	LD	Cell cycle
CDC25C	NDC	LD	Cell cycle
HIST1H1B	NDC	LD	DNA binding
RPA2	NDC	LD	DNA replication
RNASEH2A	NDC	LD	DNA replication
RFC2	NDC	LD	DNA replication
PRIM1	NDC	LD	DNA replication
ORC1L	NDC	LD	DNA replication
C10ORF119	NDC	QLVD	DNA replication
TIMM13	NDC	QLVD	Intracellular protein transport
NUP50	NDC	QLVD	Intracellular protein transport
RNF40	NDC	QLVD	Ion binding
POP5	NDC	LD	Metabolism
ALAD	NDC	LD	Metabolism
AKR1B10	NDC	LD	Metabolism
KIF22	NDC	LD	Nuclear transport
VRK1	NDC	LD	Protein binding
GFAP	NDC	QLVD	Protein binding
ANP32E	NDC	LD	Protein binding
LIG1	NDC	LD	Response to DNA damage stimulus
RAVER2	NDC	LD	RNA binding
NOL5A	NDC	QLVD	RNA binding
RSL1D1	NDC	QLVD	RNA processing
HNRNPU	NDC	LD	RNA splicing
EXOSC2	NDC	LD	rRNA processing
RHEBL1	NDC	LD	Signal transduction
PTGS1	NDC	LD	Stress/Immune response
HSPA8	NDC	QLVD	Stress/Immune response
FEN1	NDC	LD	Stress/Immune response
SMARCA5	NDC	LD	Transcription

Table 3.3 (Continued)

CARHSP1	NDC	QLVD	Transcription; Regulation
Unknown	NDC	LD	Unknown
Unknown	NDC	LD	Unknown
Unknown	NDC	LD	Unknown
Unknown	NDC	LD	Unknown
Unknown	NDC	LD	Unknown
Unknown	NDC	LD	Unknown
LOC643509	NDC	LD	Unknown
ANLN	NDD	LD	Actin binding
POSTN	NDD	LD	Cell adhesion
CDC2	NDD	QLCD	Cell cycle
CCNB3	NDD	LD	Cell cycle
CCNB1	NDD	QLCD	Cell cycle; G2/M
SMC4	NDD	QLCD	Cell cycle; M
KIF11	NDD	QLCD	Cell cycle; M
CDCA8	NDD	QLCD	Cell cycle; M
AURKB	NDD	LD	Cell cycle; M
AURKA	NDD	QLCD	Cell cycle; M
ESCO2	NDD	LD	Cell cycle; S
TPX2	NDD	QLCD	Cell proliferation
HIST1H2BJ	NDD	QLCD	Defense response
HIST2H2AC	NDD	LD	DNA binding
HBE1	NDD	QLCD	Oxygen transport
SULF1	NND	LD	Apoptosis
NID2	NND	LD	Cell adhesion
TFDP1	NND	LD	Cell cycle
PPP1CA	NND	LD	Cell cycle
CDC7	NND	LD	Cell cycle
CDK4	NND	LD	Cell cycle; G1/S
CDK2	NND	LD	Cell cycle; G2/M
PBK	NND	QLCD	Cell cycle; M
NUSAP1	NND	QLCD	Cell cycle; M
DLG7	NND	QLCD	Cell cycle; M
CETN2	NND	LD	Cell cycle; M
C11ORF31	NND	LD	Cell redox homeostasis
B3GNT5	NND	LD	Central nervous system development
VAR5	NND	LD	Defense response
RNASEH2B	NND	LD	DNA replication
RFC4	NND	LD	DNA replication
NASP	NND	LD	DNA replication
COL4A2	NND	LD	ECM component
COL4A1	NND	LD	ECM component
COL3A1	NND	LD	ECM component
COL1A2	NND	LD	ECM component
FBN2	NND	LD	Extracellular matrix structural constituent

Table 3.3 (Continued)

DNAJC9	NND	LD	Heat shock protein binding
NUP43	NND	LD	Intracellular protein transport
NUP107	NND	LD	Intracellular protein transport
KPNA2	NND	QLCD	Intracellular protein transport
FTH1_2	NND	LD	Iron homeostasis
TKTL2	NND	LD	Metabolism
FAH	NND	LD	Metabolism
DUT	NND	LD	Metabolism
ALDH6A1	NND	LD	Metabolism
CYP27C1	NND	QLCD	Metal ion binding
LMNB1	NND	LD	Nuclear structural molecule
KPNB1	NND	LD	Nuclear transport
IPO9	NND	LD	Nuclear transport
IVD	NND	LD	Oxidation reduction
TYMS	NND	QLCD	Phosphoinositide-mediated signaling
NEIL3	NND	LD	Response to DNA damage stimulus
CHAF1A	NND	LD	Response to DNA damage stimulus
HSD17B2	NND	LD	Response to retinoic acid
U2AF2	NND	QLVD	RNA binding
SNRP70	NND	QLVD	RNA binding
SFRS14	NND	LD	RNA binding
SFRS1	NND	QLCD	RNA binding
RBMX	NND	QLCD	RNA binding
NHP2L1	NND	LD	RNA binding
HNRPH2	NND	QLCD	RNA binding
HNRNPL	NND	QLCD	RNA binding
RANBP1	NND	QLVD	Signal transduction
SSTR5	NND	LD	Somatostatin receptor activity
CXCR4	NND	LD	Stress/Immune response
PTX3	NND	LD	Stress/Immune response
PARP1	NND	LD	Stress/Immune response
APEX1	NND	QLCD	Stress/Immune response
ABCF1	NND	LD	Stress/Immune response
SLTM	NND	LD	Transcription
SAFB	NND	QLCD	Transcription
NCOA5	NND	LD	Transcription
MYEF2	NND	LD	Transcription
DNMT1	NND	LD	Transcription
BAZ1B	NND	LD	Transcription
BAZ1A	NND	LD	Transcription
LMX1B	NND	LD	Transcription factor
HMGB2	NND	QLCD	Transcription factor
MED24	NND	LD	Transcription/Thyroid hormone receptor binding
Unknown	NND	QLCD	Unknown
Unknown	NND	LD	Unknown

Table 3.3 (Continued)

Unknown	NND	QLVD	Unknown
Unknown	NND	QLVD	Unknown
Unknown	NND	QLCD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	QLCD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	QLCD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	QLCD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	LD	Unknown
Unknown	NND	LD	Unknown
NLE1	NND	QLVD	Unknown
CDKN2AIPNL	NND	QLVD	Unknown
TAGLN	NNU	QLVU	Actin binding
MYO1B	NNU	LU	Actin binding
GMFG	NNU	LU	Actin binding
PDCD4	NNU	QLVU	Apoptosis
DAP	NNU	LU	Apoptosis
IFIT5	NNU	QLVU	Binding
F11R	NNU	LU	Cell adhesion
COL12A1	NNU	LU	Cell adhesion
FRK	NNU	LU	Cell cycle
CCNG1	NNU	QLVU	Cell cycle
FHL1	NNU	LU	Cell differentiation
ANXA1_3	NNU	LU	Cell differentiation
MX1	NNU	LU	Defense response
THEM2	NNU	LU	Hydrolase activity
NAPG	NNU	QLVU	Intracellular protein transport
CHPT1	NNU	LU	Lipid metabolism
LAMP1	NNU	LU	Lysosomal exocytosis
UAP1L1	NNU	LU	Metabolism
LTB4DH	NNU	LU	Metabolism
IDH3A	NNU	LU	Metabolism
HEXA	NNU	LU	Metabolism
CYP2A13	NNU	LU	Oxidation reduction
PRL	NNU	LU	Pituitary hormone

Table 3.3 (Continued)

A2M	NNU	LU	Protease inhibitor
VPS24	NNU	LU	Protein binding
USP2	NNU	LU	Protein binding
RIOK3	NNU	LU	Protein binding
PLEKHB2	NNU	LU	Protein binding
PCOLCE2	NNU	LU	Protein binding
FKBP2	NNU	QLVU	Protein binding
CNPY2	NNU	LU	Protein binding
STXBP3	NNU	LU	Protein transport
CTSK	NNU	LU	Proteolysis
PAK1	NNU	LU	Signal transduction
TIAM1	NNU	LU	Signal transduction
RRBP1	NNU	LU	Signal transduction
RAB7A	NNU	LU	Signal transduction
PDK2	NNU	LU	Signal transduction
NUPR1	NNU	LU	Signal transduction
MARCO	NNU	QLVU	Signal transduction
RPE65	NNU	LU	Stimulus response
SEPP1	NNU	LU	Stress/Immune response
CARTPT	NNU	LU	Stress/Immune response
ASL	NNU	LU	Stress/Immune response
FAM79A	NNU	LU	Synaptic vesicle component
VIT	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	QLVU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	QLVU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	QLVU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown

Table 3.3 (Continued)

Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	QLVU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	QLVU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
Unknown	NNU	LU	Unknown
TMEM128	NNU	LU	Unknown
TM6SF1	NNU	LU	Unknown
DSCR3	NNU	LU	Vacuolar transport
ZADH2	NNU	LU	Zinc ion binding
SERPINB2	NUC	LU	Anti-apoptosis
TIMP1	NUC	LU	Cell maturation
ISG15	NUC	LU	Cell-cell signaling
THNSL2	NUC	QLCU	Metabolism
MTHFD2	NUC	LU	Metabolism
GLUL	NUC	QLVU	Metabolism
ADH5	NUC	QLCU	Metabolism
ADH1C	NUC	QLCU	Metabolism
ACOX3	NUC	QLCU	Metabolism
RARRES1	NUC	LU	Negative regulation of cell proliferation
CYP2D6	NUC	LU	Oxidation reduction
DYNC1I1	NUC	LU	Protein binding
CRYAB	NUC	LU	Signal transduction
MR1	NUC	LU	Stress/Immune response
HSPA8_1	NUC	LU	Stress/Immune response
GPX1	NUC	LU	Stress/Immune response
Unknown	NUC	LU	Unknown
Unknown	NUC	LU	Unknown
Unknown	NUC	LU	Unknown
Unknown	NUC	QLCU	Unknown
Unknown	NUC	LU	Unknown
Unknown	NUC	LU	Unknown
Unknown	NUC	LU	Unknown
Unknown	NUC	LU	Unknown
Unknown	NUC	LU	Unknown
Unknown	NUC	LU	Unknown
Unknown	NUC	LU	Unknown
Unknown	NUC	LU	Unknown
CDKN1B	NUU	QLVU	Cell cycle; G1/S
GPNUMB	NUU	QLVU	Cell differentiation

Table 3.3 (Continued)

ALDH1A1	NUU	QLVU	Metabolism
NR3C2	NUU	LU	Transcription factor
Unknown	NUU	LU	Unknown
NEK3	UCC	QLCU	Cell cycle; M
ZFAND5	UCC	QLCU	Ion binding
Unknown	UCC	QLCU	Unknown
Unknown	UCC	QLCU	Unknown
Unknown	UCC	QLCU	Unknown
Unknown	UCC	QC	Unknown
Unknown	UCC	LU	Unknown
Unknown	UCC	QLCU	Unknown
HNRNPA2B1	UDC	QC	RNA binding
Unknown	UUU	LU	Unknown

The column “Pattern” describes the gene expression patterns on days 56, 70, and 84 post hatching and the column “Model” describes the gene expression patterns on days 42, 56, 70, and 84 post hatching for genes uniquely expressed by ≥ 1.5 -fold in *A. t. tigrinum*.

Table 3.4: qPCR verification of *A. mexicanum* DEGs identified via microarray at four early developmental time points.

Gene Name	Microarray 56 DPH	Q-RT-PCR 56 DPH	Microarray 70 DPH	Q-RT-PCR 70 DPH	Microarray 84 DPH	Q-RT-PCR 84 DPH
GLUL	1.118 ± 0.796	1.0803 ± 0.739	1.254 ± 0.175	2.115 ± 0.041	1.295 ± 0.186	2.174 ± 0.049
HBA2	1.165 ± 0.023	1.223 ± 0.032	1.194 ± 0.082	-1.015 ± 0.762	-1.076 ± 0.005	-2.274 ± 0.127
HBD	1.032 ± 0.701	25.472 ± 4.597	1.288 ± 0.111	70.332 ± 12.370	1.393 ± 0.041	137.976 ± 12.618
HBE1	1.244 ± 0.025	1.954 ± 0.092	1.352 ± 0.022	2.905 ± 0.281	1.284 ± 0.011	2.107 ± 0.258
HBG1	11.49 ± 2.409	18.034 ± 3.960	34.939 ± 4.563	98.65 ± 29.327	49.977 ± 1.540	157.982 ± 15.856
JARID2	-1.485 ± 0.133	-1.421 ± 0.176	1.051 ± 0.709	1.049 ± 0.721	-1.326 ± 0.063	-1.237 ± 0.841
NR3C2	1.117 ± 0.781	1.114 ± 0.047	-1.108 ± 0.077	1.425 ± 0.079	-1.072 ± 0.722	1.611 ± 0.072
POMC	-1.172 ± 0.887	-1.052 ± 0.993	1.244 ± 0.048	1.547 ± 0.126	1.617 ± 0.102	2.1523 ± 0.105
TUBB2C	-1.014 ± 0.675	-1.375 ± 0.109	-1.023 ± 0.681	-1.549 ± 0.020	-1.062 ± 0.009	-1.64 ± 0.089

Mean fold change of each time point, relative to the mean *A. mexicanum* 42 DPH. Consequently, positive values are up regulated relative to 42 DPH samples and negative values are down regulated relative to 42 DPH samples. All values are ± standard error corresponding time point.

Table 3.5: qPCR verification of *A. t. tigrinum* DEGs identified via microarray at four early developmental time points.

Gene Name	Microarray 56 DPH	Q-RT-PCR 56 DPH	Microarray 70 DPH	Q-RT-PCR 70 DPH	Microarray 84 DPH	Q-RT-PCR 84 DPH
GLUL	1.156 ± 0.100	1.072 ± 0.793	1.686 ± 0.088	1.974 ± 0.149	2.610 ± 0.086	2.600 ± 0.135
HBA2	-5.845 ± 0.230	0.326 ± 0.043	-7.019 ± 1.027	-0.409 ± 0.084	-9.970 ± 0.179	-12.884 ± 9.600
HBD	3.975 ± 0.814	5.590 ± 1.025	7.893 ± 0.683	10.864 ± 0.526	7.379 ± 0.377	8.967 ± 0.390
HBE1	-1.257 ± 0.028	0.467 ± 0.049	-2.009 ± 0.188	-0.561 ± 0.096	-18.809 ± 5.002	-9.347 ± 1.808
HBG1	1.608 ± 0.049	4.471 ± 0.974	1.757 ± 0.021	7.643 ± 0.667	1.762 ± 0.014	8.306 ± 1.083
JARID2	-1.891 ± 0.049	-0.813 ± 0.068	1.001 ± 0.769	0.968 ± 0.079	1.342 ± 0.091	1.266 ± 1.164
NR3C2	1.298 ± 0.102	1.827 ± 0.032	1.776 ± 0.121	2.827 ± 0.171	2.766 ± 0.080	3.75 ± 0.460
POMC	1.293 ± 0.75	1.617 ± 0.159	1.398 ± 0.135	1.652 ± 0.311	1.439 ± 0.139	1.790 ± 0.423
TUBB2C	1.185 ± 0.074	1.462 ± 0.937	1.131 ± 0.763	1.998 ± 0.142	1.028 ± 0.701	3.407 ± 1.531

Mean fold change of each time point, relative to the mean *A. t. tigrinum* 42 DPH. Consequently, positive values are up regulated relative to 42 DPH samples and negative values are down regulated relative to 42 DPH samples. All values are ± standard error corresponding time point.

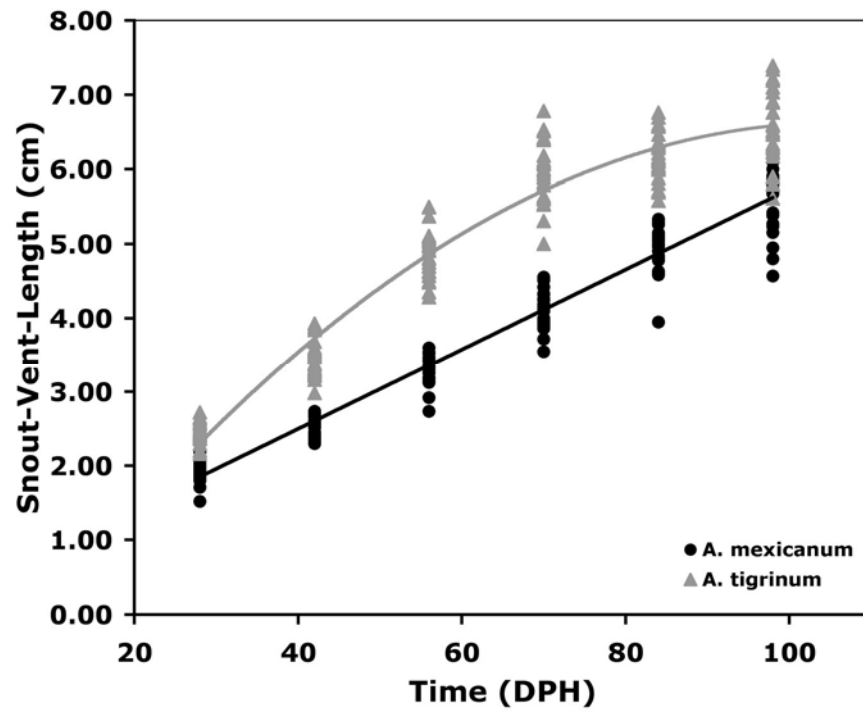


Figure 3.1: *A. mexicanum* and *A. t. tigrinum* growth rate. Individual SVL measured at time of tissue collection.

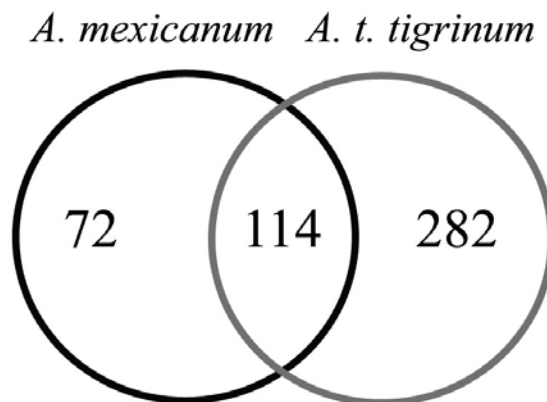


Figure 3.2: Venn diagram showing the DEGs identified unique in *A. mexicanum* and *A. t. tigrinum*, and the DEGs in common between the two species

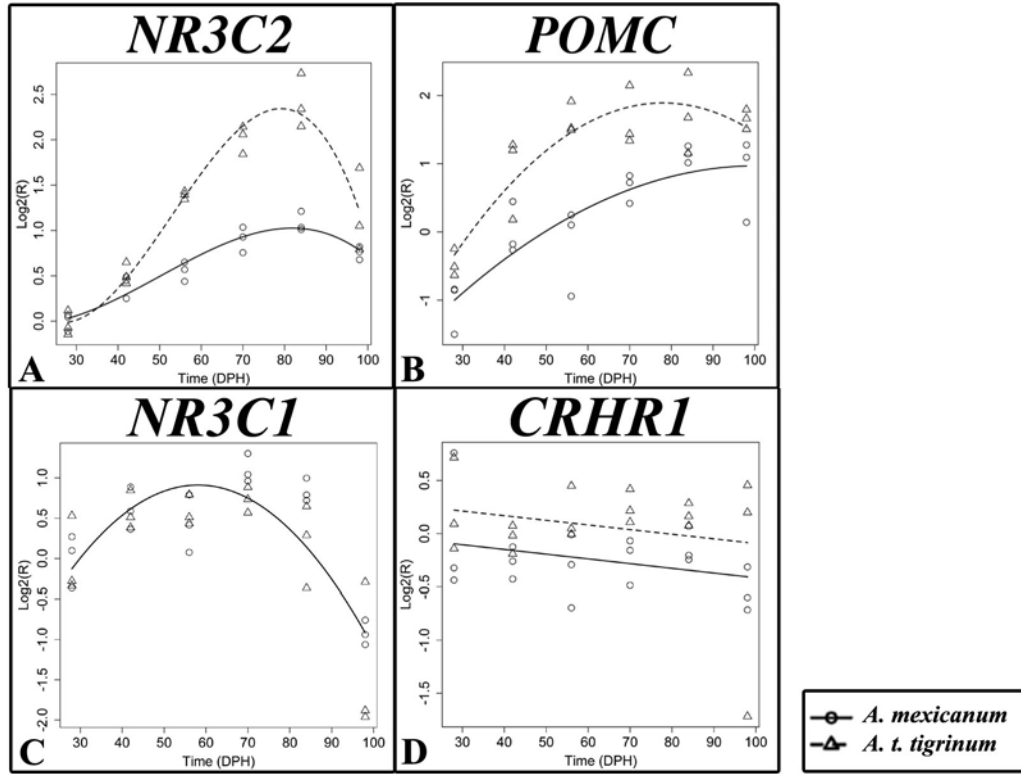


Figure 3.3: Expression profiles generated via qPCR for all 6 developmental time points investigated. All genes except *NR3C1* are statistically different between *A. mexicanum* and *A. t. tigrinum*. (A) Nuclear receptor subfamily 3 group C member 2, (B) Proopiomelanocortin, (C) Nuclear receptor subfamily 3 group C member 1, (D) Corticotrophin releasing hormone receptor 1.

CHAPTER FOUR: DISCUSSION

The Mexican axolotl has long been considered a hopeful monster because it exhibits an adaptive and derived mode of development, often referred to as paedomorphosis in the literature, that may have evolved as a result of few physiological or genetic changes of a metamorphic ancestor (Goldschmidt, 1940; Gould, 1977a). This study used a comparative genomic approach to gain new insight about the developmental basis of paedomorphosis in the Mexican axolotl as well as metamorphosis in *A. t. tigrinum*. Transcripts were sampled from these species at comparable times during the larval period to gain perspective about the development and function of brain regions that are known to regulate the secretion of metamorphic hormones. The results show both similarities and species-specific differences in the expression of transcripts. Gene expression patterns were more similar between *A. mexicanum* and *A. t. tigrinum* at early stages of larval development. At later stages of development, more uniquely expressed genes were identified from both species, with considerably more unique genes identified from *A. t. tigrinum*. These patterns were not unexpected. There are many aspects of brain development and function that are shared broadly among vertebrates (Reichert, 2009). Conversely, there has also been ample time for *A. mexicanum* and *A. t. tigrinum* to evolve species-specific transcriptional differences over evolutionary time. Indeed, the transcriptome of the vertebrate brain has considerable potential for rapid evolution (Aubin-Horth et al., 2005; Mank et al., 2007; Whitfield et al., 2003). However, a key finding of this study is that very early transcriptional differences were observed between the species for genes with HPI axis functional annotations. Divergence of HPI axis function during early development correlates with the divergence of metamorphic and paedomorphic transcriptional programs exhibited by these species. These findings do not support the developmental equivalence hypothesis, that paedomorphic and metamorphic larvae exhibit the same pattern of development until a metamorphic developmental switch is turn-on late in the larval period. Rather, metamorphic and paedomorphic developmental modes are associated with different transcriptional programs in the brain, which deviate early in ontogeny well before the onset of morphological metamorphosis. Below I discuss further gene expression patterns and gene functions that were identified

in this study and conclude by discussing the differential regulation of the HPI axis between *A. mexicanum* and *A. t. tigrinum*.

A similar expression pattern was observed for many DEGs of the shared and unique *A. t. tigrinum* gene lists. There was a general decrease in expression of transcription factors and cell cycle genes during larval development. This pattern suggests inhibition of cellular proliferation and perhaps, fate specification within certain neuronal cell populations. It is possible that many of the genes that function in the specification and proliferation of neuronal cell types are similarly expressed during development, in both metamorphic and paedomorphic salamanders. Genes associated with vertebrate brain development such as *(sex determining region Y)-box3* (Alatzoglou et al., 2009), *msh homeobox homolog 1* (Bach et al., 2003), and *neuropeptide Y* (Agasse et al., 2008), were differentially regulated in *A. mexicanum* and *A. t. tigrinum*; they also exhibited the same down-regulated gene expression pattern. Other genes showed increases in transcript abundance during the larval period in both species, a pattern that is also seen in other vertebrates. For example, *clusterin* is expressed at low levels in the central nervous system during embryogenesis in mice before showing an increase in expression as postnatal life progresses (Charnay et al., 2008). Many aspects of brain development and function are highly conserved among vertebrates. If these functions depend upon conserved patterns of gene transcription, then similarities are expected whether a salamander follows a metamorphic or paedomorphic life history.

Although many genes exhibited the same expression pattern between *A. mexicanum* and *A. t. tigrinum*, 4x more genes were identified uniquely from *A. t. tigrinum*. It is possible that many of the unique gene expression changes identified from *A. t. tigrinum* are early transcriptional responses that are necessary for later metamorphic transcriptional events. Two lines of evidence support this idea – 1) More genes were uniquely expressed in *A. t. tigrinum* and the majority showed significant fold-level changes between the two, latest sample times. It is known from microarray analyses of Mexican axolotls that thousands of genes are differentially expressed during TH-induced metamorphosis and cryptic gene expression is observed prior to morphological metamorphosis (Page et al., 2007; 2008; 2009). 2) Genes and gene functions were identified that are likely associated with metamorphosis. First, several genes (*e.g. dnmt1*,

baz1b, *baz1a*, *smarca5*) code for proteins that modify chromatin and repress gene expression. These genes were down regulated, suggesting a transcriptional mechanism that relieves gene repression and therefore promotes gene activation. It is well established that insect and amphibian metamorphosis is associated with chromatin remodelling events that reprogram cells to activate new transcriptional programs (Badenhorst et al., 2005; Carrera et al., 2000; Miotto et al., 2006; Sachs and Shi, 2000; Sachs et al., 2000). Second, several genes involved in nuclear signaling and hormone pathways (e.g. *sstr5*, *pomc*, *mr1*, *nr3c2*, *prl*, *pak1*, *jarid2*, *nupr1*) increased in expression during larval development. These unique *A. t. tigrinum* genes comprise the first list of candidate metamorphic response genes from the brain of a salamander.

An important finding of the study is that many genes are uniquely expressed in larvae of paedomorphic axolotls at the same time that gene expression events increase in *A. t. tigrinum*. This suggests that paedomorphosis is associated with a unique pattern of brain development that maybe adaptive for an aquatic life history. In support of this idea, *A. mexicanum* showed increased expression of genes that are implicated in brain development, including extracellular matrix molecules (Brenneke et al., 2004; Lively and Brown, 2008), apoptotic factors (Hamburger and Oppenheim, 1982; Mooney and Miller, 2000), and proteins that mediate cell-cell (Domínguez del Toro et al., 1997; Shingo et al., 2001) and cell-matrix interactions (Brenneke et al., 2004; Rønn et al., 1998). Alternatively, many of the unique *A. mexicanum* genes may have evolved as a result of neutral evolutionary processes. This seems possible because the *A. mexicanum* in this experiment came from the Ambystoma Genetic Stock Center, which maintains a highly inbred population under domesticated conditions. However, it is curious that four genes that are associated with mammalian brain pathologies were uniquely up regulated in *A. mexicanum*. These include: (1) *ctss*, an apoptotic factor that provides a marker for aging and related pathologies in the mammalian central nervous system (Hu et al., 2005), (2) *cd69*, a lymphocyte marker that is expressed highly in Alzheimers patients (Kusdra et al., 2000), (3) *ogn* is up regulated in some human pituitary tumors and appears to be under the transcriptional control of *pit1*, which is associated with the differentiation and function of cells that secrete pituitary hormones (Hu et al., 2005), and (4) *dcn* is differentially expressed in transgenic mice that over-express CRH (Peeters et al., 2004).

Further investigation of these loci is needed to resolve their adaptive and/or pathological significance in the paedomorphic axolotl.

I conclude by discussing one of the most significant findings of the study, the differential expression of HPI axis genes between *A. t. tigrinum* and *A. mexicanum*. The secretion of neuroendocrine and endocrine factors by the HPA/I axis is pivotal in directing development and regulating stress responses in vertebrates (Denver, 1999). Generally speaking, the vertebrate hypothalamus responds to intrinsic and extrinsic stimuli by releasing CRH and this acts at the level of the pituitary to stimulate secretion of POMC (ACTH), and ACTH subsequently stimulates the adrenal gland to release glucocorticoids (GC). Adrenal GC feeds back on the brain to regulate the HPA axis by binding to glucocorticoid (GR) and mineralocorticoid (MR) receptors (McEwen et al., 1986), which then translocate to the nucleus and direct transcription of target genes. The distribution of GR and MR in the brain is variable and overlapping, and this diversity is thought to be biologically significant as it varies among brain regions that mediate different functions. For example, functional studies show that targeted alteration of GR and MR transcription in mammalian brain regions alters the behavior and stress response of mice (Rozenboom et al., 2007). These and earlier studies support the idea that variation in behavior and physiology are associated with variable GR : MR ratios in the brain (De Kloet, 1991; Barbazanges et al., 1996; De Kloet et al, 1998).

In amphibians, the HP-Interrenal axis (the amphibian interrenal glands are homologous to the mammalian adrenals) regulates the onset of metamorphosis. Studies have shown that environmental factors can delay or accelerate larval growth by modulating the timing of CRH release from the hypothalamus (Amiel-Tison et al., 2004). Interestingly in amphibians, CRH not only stimulates POMC secretion, it also stimulates thyroid-stimulating hormone (TSH) release and thyroid hormone signaling (TH). Whereas TH is necessary and sufficient to induce metamorphosis, activation of the HPI axis under stressful conditions releases GC, and GC synergizes with TH to accelerate metamorphosis. For example, stress that is associated with habitat deterioration can accelerate development and cause an earlier onset timing of metamorphosis by stimulating CRH and corticosteroid release (Denver, 1999). Conversely, larvae capable of metamorphosis can delay metamorphosis in favorable growth environments by

producing substances that antagonize the HPI axis (Norris, 1978). It is likely that cross talk between HPI and HPT axes is an evolutionarily conserved mechanism that is common to all vertebrates, including humans. For example, it is known that handling stress increases the secretion of thyroid hormone in rats and it is hypothesized that stress-induced thyroid hormone secretion is associated with preterm births and accelerated neurological development in humans (Amiel-Tison et al., 2004).

If GR : MR ratios similarly affect physiological and behavioral processes during salamander development, my results suggest the brains of larval *A. mexicanum* and *A. t. tigrinum* begin to diverge functionally around 42 dph. At this time, *crhr1* and *pomc* transcript abundances were higher in *A. t. tigrinum* larval brains, and mineralocorticoid receptor (*nr3c2*) abundances began to diverge. After this time, *nr3c2* transcripts increased dramatically in *A. t. tigrinum* but only increased slightly in *A. mexicanum*. Interestingly, glucocorticoid receptors (*nr3c1*) showed a similar up-regulated expression profile in both species. Taken together and assuming that my transcriptional estimates correlate with HPI protein activity (Ali et al., 2005), GR : MR ratios and POMC levels are predicted to differ between the brains of larval *A. mexicanum* and *A. t. tigrinum*. These differences may help explain why a tiger salamander undergoes a metamorphosis and an axolotl undergoes paedomorphosis. For example, it is possible that the HPI axis is not maintained at a sufficiently high enough level in *A. mexicanum* to stimulate release of TSH from the anterior pituitary. Consequently, the thyroid gland is not stimulated to secrete thyroid hormone and metamorphosis is not initiated.

In conclusion, this study shows that metamorphic and paedomorphic modes of development are associated with different transcriptional programs in the brain, and these programs diverge during early larval development. These early transcriptional differences include genes that function in the HPI axis. Future studies investigating transcriptional differences through the duration of metamorphosis are needed to investigate whether the early metamorphic response genes and genes that function in the HPI axis continue to show transcriptional differences between metamorphic and paedomorphic salamanders. Furthermore, studies of additional metamorphic and paedomorphic salamanders are needed to disentangle species-specific gene expression responses from those that distinguish metamorphic and paedomorphic modes of development

APPENDIX I
A. *mexicanum* significant genes

Probe-set	Gene Symbol	Model	Pattern	Function	42 DPH	56 DPH	70 DPH	84 DPH
SRV_11152_at	ACTG2	LU	NNU	Actin binding	601.63	726.87	884.34	963.68
SRV_00461_a_at	ADH4	LU	NUC	Unknown	333.85	473.30	509.36	669.62
SRV_03523_at	AKAP8L	LD	NND	Ion/protein binding	94.83	84.84	76.18	55.34
SRV_00124_a_at	ALAS2	QLCU	UCC	Stress/Immune response	231.73	518.14	629.45	645.65
SRV_04261_at	ALG13	QLCU	NNU	Metabolism	849.83	1,098.17	1,225.16	1,304.82
SRV_00752_a_at	ANG	LU	NNU	Cell differentiation	393.86	415.26	526.62	715.55
SRV_00492_a_at	ANXA1	LU	NNU	Cell differentiation	775.26	1,053.75	1,004.19	1,235.83
SRV_00501_a_at	ANXA1	LU	NUC	Cell differentiation	61.46	83.16	130.06	176.21
SRV_00772_at	ANXA5	LU	NNU	Signal transduction	1,457.98	1,995.18	2,043.11	2,529.24
SRV_00130_a_at	APOE	LU	NUC	Stress/Immune response	1,261.69	1,543.95	2,310.70	2,980.32
SRV_01139_a_at	ATP6V1C1	LU	NNU	Metabolism	2,092.70	2,475.07	2,676.99	3,176.43
SRV_02056_a_at	B2M	LU	NNU	Stress/Immune response	553.30	585.96	641.96	1,316.47
SRV_01149_at	BHMT	LU	NNU	Metabolism	1,625.31	1,777.31	2,096.04	2,712.06
SRV_10156_s_at	C10ORF54	LU	NNU	Receptor activity	149.45	171.96	183.74	228.56
SRV_05942_at	C6ORF115	QLVD	NND	Cell cycle; M	2,616.31	2,096.30	1,891.05	1,630.24
SRV_01164_a_at	CAPNS1	LU	NNU	Apoptosis	280.50	337.01	400.04	492.91
SRV_01167_a_at	CAT	LU	UCC	Stress/Immune response	227.33	434.74	637.28	841.79
SRV_00799_at	CCNA2	QLVD	NDC	Cell cycle: Checkpoint	467.87	393.97	292.90	290.85
SRV_03357_at	CD69	QLVU	NNU	Signal transduction	185.72	190.93	240.75	347.70
SRV_02274_at	CD74	LU	NUC	Stress/Immune response	328.03	419.72	499.41	670.28
SRV_01173_at	CD9	LU	NUC	Signal transduction	2,900.01	3,933.36	4,927.11	5,595.91
SRV_00806_a_at	CDC20	QLVD	NDC	Cell cycle; M	748.44	631.27	485.68	509.88
SRV_01183_at	CDC25A	LD	NND	Cell cycle; G1/S	320.70	309.32	245.71	207.42
SRV_00804_at	CDC6	LD	NND	Cell cycle; DNA replication	296.09	244.01	206.68	194.03
SRV_00319_at	CDH1	QLCU	NUC	Cell-cell adhesion	781.86	1,088.62	1,496.65	1,747.87

SRV_00807_at	CDK9	LD	NND	Regulation of cell cycle	321.51	273.39	224.17	213.83
SRV_00813_at	CHD4	LD	NND	DNA metabolism	2,048.59	1,705.95	1,373.22	1,235.58
SRV_00815_a_at	CHEK1	LD	NDC	Cell cycle; DNA damage	238.05	191.12	147.40	152.08
SRV_01199_a_at	CLU	LU	NUC	Cell differentiation	1,684.21	2,087.69	2,742.32	2,967.53
SRV_04978_a_at	CNDP1	LU	NNU	Peptidase activity	156.87	203.72	207.20	240.37
SRV_04977_at	CNDP2	LU	UCC	Dipeptidase activity	1,448.17	2,293.33	2,874.42	2,926.32
E_at	COL1A1	QLCU	NUC	ECM component	3,480.65	4,915.72	5,496.45	5,164.84
SRV_01205_at	COL2A1	LD	NDC	Organ development	769.70	577.76	489.00	374.93
SRV_00149_a_at	CST3	LU	NUC	Tissue remodeling	1,179.41	1,626.13	2,507.95	3,194.82
SRV_02071_a_at	CTSS	QLCU	NUC	Apoptosis	597.79	811.06	965.36	942.45
SRV_14170_a_at	DCN	QLCU	NUC	Organ morphogenesis	2,422.97	3,131.55	3,937.59	4,110.88
SRV_02182_at	DDX5	LD	NND	ATP binding	2,802.20	2,297.67	2,158.48	1,743.89
SRV_04967_a_at	DGAT2	QLCU	NNU	Metabolism	230.97	315.87	345.15	378.38
SRV_03221_a_at	DMBT1	LU	NNU	Immune system	88.20	111.90	105.58	138.73
SRV_00001_at	DNTT	LU	NUC	DNA replication	130.65	146.26	217.02	275.83
SRV_01254_a_at	DPT	LU	NUC	ECM component	65.22	79.55	102.06	115.25
SRV_05635_a_at	EIF4G1	LD	NND	mRNA processing	907.96	801.94	686.69	581.32
SRV_02483_at	EWSR1	QLVD	NDC	RNA binding	1,374.09	1,092.29	825.33	865.35
SRV_00949_at	FAAH	QLCU	UCC	Fatty acid hydrolysis	320.36	525.25	651.55	738.35
SRV_02451_at	FGFBP1	LU	NUC	FGF signaling	741.54	919.45	1,123.50	1,207.90
SRV_00954_at	FLNA	LD	NND	Actin binding	276.49	242.85	188.36	162.30
SRV_00167_a_at	FTH1	QLCU	UUC	Iron homeostasis	508.26	790.83	1,257.28	1,462.84
SRV_02740_a_at	FXRD3	LU	NNU	Ion transport	1,535.15	1,921.61	2,191.64	2,546.63
SRV_00531_at	GSTP1	LU	NNU	Neuroprotection	1,052.25	1,269.73	1,488.83	1,589.23
SRV_02502_a_at	H3F3B	LD	NDC	DNA metabolism	4,253.43	3,351.98	2,613.36	2,541.83
SRV_00424_x_at	HBA2	QLCU	NUC	Oxygen transport	16,217.44	21,738.83	24,425.18	22,686.13
SRV_00397_s_at	HBA2	QLCU	UUC	Oxygen transport	1,007.14	6,906.59	17,597.86	23,247.71
SRV_00375_s_at	HBD	QLCU	UUC	Oxygen transport	1,023.81	7,218.77	16,314.62	19,887.49
SRV_00434_x_at	HBG1	QLCU	UUC	Oxygen transport	368.01	4,713.42	14,672.12	20,333.49
SRV_00037_at	HBZ	QLCD	NDD	Oxygen transport	6,486.13	6,488.05	4,004.08	1,384.99

SRV_03506_at	HEBP2	LU	NUC	Apoptosis	402.80	540.81	886.15	1,018.86
SRV_00193_at	IDUA	LU	NNU	Hydrolysis	176.24	224.12	238.74	307.67
SRV_04367_a_at	IGLL1	LU	NUC	Stress/Immune response	81.65	107.28	122.56	162.49
SRV_07449_x_at	ILF3	QLVD	NDC	Cell cycle	3,313.07	2,614.92	2,176.14	1,979.68
SRV_05450_a_at	ITGB1BP3	QLCU	NUC	Metabolism	854.14	1,031.69	1,324.35	1,196.67
SRV_01942_a_at	KHSRP	LD	NND	Transcription	2,712.86	2,265.98	1,853.27	1,724.36
SRV_03588_at	KIAA0101	LD	NDC	Cell cycle; S	1,235.82	1,067.75	717.86	796.64
SRV_03695_at	KIAA0776	LU	NNU	Protein binding	401.62	458.94	520.42	632.75
SRV_02578_at	KIFC1	QLVD	NDC	Cell cycle; M	860.09	728.65	558.83	592.21
SRV_00344_at	KRT5	QLCU	NUC	Cytoskeleton	3,679.95	4,793.22	5,938.75	5,785.51
SRV_02580_a_at	LAMA1	LD	NDC	ECM component	239.77	168.42	135.46	107.86
SRV_01387_at	LGALS1	LU	NUC	Cell differentiation	2,329.93	3,158.78	3,904.79	4,936.15
SRV_02816_a_at	LGALS4	LU	NNU	Cell adhesion	2,024.25	2,377.17	2,806.61	3,110.15
SRV_04989_at	LMNB2	LD	NDC	Structural molecule activity	763.49	760.33	490.51	450.20
SRV_09752_at	LOC390940	LU	NUU	Unknown	111.51	139.53	189.94	410.07
SRV_07838_a_at	LOH11CR2A	QLCU	NUC	Cell cycle	367.10	473.47	646.15	706.19
SRV_01409_at	MAD2L1	QLVD	NDC	Cell cycle; M	337.92	248.86	194.53	208.65
SRV_01414_at	MCM3	QLVD	NDC	Cell cycle; DNA replication	664.68	529.95	420.86	398.47
SRV_05623_at	MCM4	LD	NDC	DNA metabolism	1,257.98	1,018.62	838.56	737.13
SRV_02725_a_at	MCM6	QLVD	NND	Cell cycle; S	1,015.36	812.83	709.42	662.25
SRV_02727_at	MCM7	QLVD	NDC	Cell cycle; DNA replication	1,508.01	1,215.96	944.69	901.78
SRV_04611_a_at	MLF1	QLVU	NNU	Cell differentiation	271.67	272.98	338.82	431.31
SRV_11417_a_at	MMP1	LD	DCC	Tissue remodeling	105.33	69.32	62.29	51.05
SRV_00011_at	MSX1	LD	NDC	Transcription	211.30	157.27	122.18	96.06
SRV_03513_at	MTCH1	LU	NNU	Apoptosis	293.06	406.09	388.45	481.61
SRV_00080_at	NPY	LU	NNU	Stimulus response	679.46	834.98	970.60	1,091.37
SRV_03432_s_at	OGN	LU	NUC	Growth factor activity	163.09	223.77	251.79	302.14
SRV_04532_a_at	PCTP	LU	NNU	Lipid transport	75.31	95.82	105.84	143.85
SRV_12805_at	PFAS	LD	NDC	Metabolism	101.75	92.79	61.32	71.89
SRV_01517_at	PIM1	LD	NDC	Negative regulation of apoptosis	774.80	629.60	495.91	466.27

SRV_01945_a_at	PLA2G4C	LU	NUU	Stress/Immune response	42.55	63.29	105.59	199.39
SRV_02420_at	PLK1	LD	NDC	Cell cycle; M	773.37	671.55	473.62	465.17
SRV_02067_at	PLK3	LD	NDC	Regulation of cell cycle	149.96	119.18	94.50	97.31
SRV_04514_at	POLR2L	LU	NUC	Transcription	282.82	308.29	571.78	614.75
SRV_00557_a_at	PPIC	LU	NNU	Signal transduction	394.06	437.27	535.96	621.69
SRV_04881_at	PTBP1	LD	NDC	Metabolism	1,704.28	1,522.38	1,125.44	1,042.36
SRV_02923_at	RABAC1	QLCD	NND	Protein binding	245.57	230.79	219.13	160.39
SRV_00812_a_at	RCC1	LD	NDC	Cell cycle; M	658.98	568.48	434.37	421.31
SRV_04287_at	RCC2	LD	NDC	Cell cycle; M	352.23	319.28	206.61	216.89
SRV_00638_at	RPL31	QLVD	DCC	Protein synthesis	459.49	294.45	294.54	273.36
SRV_00705_at	RRM1	LD	NDC	Cell cycle; DNA replication	2,862.54	2,319.67	1,874.25	1,704.44
SRV_00706_a_at	RRM2	QLVD	NDC	Cell cycle; DNA replication	1,843.30	1,448.19	1,004.49	1,024.78
SRV_04195_at	SEPT8	QLCU	NNU	Cell cycle	480.79	612.13	681.13	737.35
SRV_03747_a_at	SERBP1	QLCD	NND	mRNA binding	1,347.05	1,448.19	1,090.79	739.47
SRV_02418_at	SERPINB10	QLCU	NUC	Protein binding	199.00	251.06	303.16	286.48
SRV_00796_at	SERPINH1	LU	NUC	Stress/Immune response	102.38	138.14	158.71	183.97
SRV_02964_s_at	SLBP	QLVD	NDC	mRNA processing	527.55	423.98	321.62	340.51
SRV_02613_at	SOX3	LD	NDC	Transcription factor	1,448.64	1,088.80	897.75	793.53
SRV_03652_at	SPEN	QLVD	NDC	Transcription	2,410.07	1,757.68	1,574.51	1,535.99
SRV_01775_at	TEAD4	LD	NDC	Transcription	112.73	91.52	67.02	60.97
SRV_03078_a_at	TMED10	LD	NND	Protein transport	2,427.50	2,107.45	1,962.67	1,567.02
SRV_03419_at	TMEM176B	QLCU	NUC	Organ morphogenesis	622.64	888.03	1,132.04	1,456.75
SRV_01795_at	TMPO	LD	NND	Transcription	953.74	838.65	683.25	631.06
SRV_01821_at	TYROBP	LU	NNU	Stress/Immune response	136.68	161.87	201.62	222.50
SRV_03361_at	UHRF1	QLVD	NDC	Cell cycle	496.20	380.19	268.87	259.79
SRV_03360_at	UHRF2	LD	NDC	Cell cycle; Regulation	807.58	622.00	465.82	393.46
SRV_06379_at	Unknown	QLVD	DCC	Unknown	130.35	64.14	65.90	68.49
SRV_09891_at	Unknown	QLVD	DCC	Unknown	331.52	217.26	230.33	225.83
SRV_09914_at	Unknown	QLVD	DCC	Unknown	762.01	484.32	417.68	412.48
SRV_10220_at	Unknown	QLVD	DCC	Unknown	84.45	49.14	39.05	36.23

SRV_05804_at	Unknown	QLVD	DDC	Unknown	398.79	186.89	123.11	87.23
SRV_06485_a_at	Unknown	LD	NDC	Unknown	247.76	216.80	161.81	175.45
SRV_06965_at	Unknown	LD	NDC	Unknown	956.85	862.92	616.60	598.55
SRV_07119_s_at	Unknown	LD	NDC	Unknown	816.61	707.06	513.62	516.37
SRV_07203_at	Unknown	LD	NDC	Unknown	250.40	218.15	160.84	143.30
SRV_08403_a_at	Unknown	QLVD	NDC	Unknown	208.42	144.46	135.45	140.00
SRV_08476_at	Unknown	LD	NDC	Unknown	528.96	465.47	351.18	319.51
SRV_08912_a_at	Unknown	LD	NDC	Unknown	264.36	207.18	161.04	159.72
SRV_09165_at	Unknown	LD	NDC	Unknown	748.93	627.01	457.79	485.61
SRV_09327_a_at	Unknown	QLVD	NDC	Unknown	932.00	646.71	496.48	449.56
SRV_09459_at	Unknown	LD	NDC	Unknown	1,006.56	784.90	596.09	526.72
SRV_09914_x_at	Unknown	QLVD	NDC	Unknown	882.15	627.41	581.22	572.72
SRV_10495_s_at	Unknown	LD	NDC	Unknown	545.58	465.42	360.90	341.22
SRV_01943_a_at	Unknown	LD	NND	Unknown	1,428.17	1,335.25	989.26	900.17
SRV_02852_a_at	Unknown	LD	NND	Intracellular transport	93.80	69.40	68.78	56.21
SRV_03657_at	Unknown	LD	NND	Unknown	319.08	262.27	231.58	209.87
SRV_05721_at	Unknown	LD	NND	Unknown	1,360.46	1,189.45	966.30	900.76
SRV_05828_a_at	Unknown	LD	NND	Unknown	1,619.81	1,373.71	1,111.41	1,038.35
SRV_06088_a_at	Unknown	LD	NND	Unknown	322.41	240.79	231.83	208.32
SRV_06364_a_at	Unknown	LD	NND	Unknown	489.59	420.75	340.01	284.51
SRV_06667_a_at	Unknown	LD	NND	Unknown	1,026.95	850.76	785.57	660.16
SRV_06956_at	Unknown	QLVD	NND	Unknown	1,867.07	1,530.37	1,263.65	1,186.87
SRV_07004_at	Unknown	QLVD	NND	Unknown	906.07	740.02	604.01	603.33
SRV_07026_at	Unknown	LD	NND	Unknown	3,052.15	2,526.94	2,391.62	1,964.96
SRV_07269_at	Unknown	LD	NND	Unknown	500.31	418.35	356.30	292.04
SRV_07334_a_at	Unknown	LD	NND	Unknown	5,759.90	4,963.43	4,360.56	3,762.48
SRV_07521_a_at	Unknown	LD	NND	Unknown	3,521.34	3,448.08	3,037.30	2,283.80
SRV_08376_at	Unknown	LD	NND	Unknown	5,356.45	5,072.33	3,982.64	3,506.11
SRV_08473_at	Unknown	LD	NND	Unknown	552.78	529.36	411.95	359.19
SRV_08603_a_at	Unknown	LD	NND	Unknown	1,913.44	1,711.62	1,401.29	1,152.10

SRV_08712_at	Unknown	LD	NND	Unknown	315.51	255.13	224.58	195.06
SRV_08811_at	Unknown	LD	NND	Unknown	2,562.13	2,117.72	1,863.19	1,583.83
SRV_08910_at	Unknown	LD	NND	Unknown	1,618.78	1,383.28	1,181.11	970.30
SRV_09217_at	Unknown	LD	NND	Unknown	2,599.42	2,175.85	2,071.82	1,711.16
SRV_09248_at	Unknown	LD	NND	Unknown	1,288.77	1,098.84	905.06	771.88
SRV_09269_s_at	Unknown	LD	NND	Unknown	655.80	575.81	492.53	389.02
SRV_09427_at	Unknown	LD	NND	Unknown	543.70	441.87	365.77	319.86
SRV_09469_at	Unknown	QLVD	NND	Unknown	1,092.41	729.78	771.97	701.91
SRV_10204_at	Unknown	LD	NND	Unknown	920.94	798.43	689.73	608.77
SRV_10334_at	Unknown	LD	NND	Unknown	94.07	68.22	68.44	56.39
SRV_10395_at	Unknown	LD	NND	Unknown	93.50	87.96	71.61	62.02
SRV_10397_at	Unknown	LD	NND	Unknown	1,837.76	1,573.66	1,346.57	1,186.92
SRV_10442_at	Unknown	QLVD	NND	Unknown	841.52	623.05	602.89	540.53
SRV_02137_at	Unknown	LU	NNU	Unknown	122.39	175.20	175.60	208.79
SRV_06202_x_at	Unknown	LU	NNU	Unknown	648.71	655.61	836.14	1,163.80
SRV_07376_at	Unknown	LU	NNU	Unknown	440.33	568.86	618.69	878.24
SRV_07430_a_at	Unknown	LU	NNU	Unknown	65.46	82.63	93.21	99.50
SRV_07989_at	Unknown	LU	NNU	Unknown	2,090.63	2,489.54	3,073.27	3,579.54
SRV_08599_a_at	Unknown	LU	NNU	Unknown	30.09	36.42	42.50	50.36
SRV_09326_s_at	Unknown	LU	NNU	Unknown	790.26	1,097.05	1,115.47	1,905.25
SRV_09415_at	Unknown	LU	NNU	Unknown	91.18	112.56	122.25	138.89
SRV_09535_at	Unknown	LU	NNU	Unknown	203.01	238.36	298.45	384.32
SRV_09599_at	Unknown	LU	NNU	Unknown	1,634.65	1,825.84	2,450.53	2,616.29
SRV_09788_s_at	Unknown	LU	NNU	Unknown	731.47	863.41	971.11	1,128.18
SRV_09904_s_at	Unknown	LU	NNU	Unknown	525.63	687.50	667.80	793.72
SRV_09912_at	Unknown	QLVU	NNU	Unknown	282.71	296.98	304.96	430.22
SRV_09951_at	Unknown	LU	NNU	Unknown	225.76	233.21	274.81	349.09
SRV_06712_a_at	Unknown	LU	NUC	Unknown	4,564.70	6,289.54	8,318.90	9,375.35
SRV_07072_x_at	Unknown	LU	NUC	Unknown	2,195.23	2,722.38	3,297.67	3,451.81
SRV_07501_at	Unknown	LU	NUC	Unknown	97.67	134.25	153.05	199.28

SRV_07809_at	Unknown	LU	NUC	Unknown	499.21	597.13	784.63	905.50
SRV_07933_at	Unknown	LU	NUC	Unknown	497.14	654.04	783.87	865.91
SRV_09416_at	Unknown	LU	NUC	Unknown	725.02	841.88	1,124.21	1,232.90
SRV_09852_at	Unknown	LU	NUC	Unknown	1,252.42	1,404.16	1,896.98	1,897.74
SRV_10340_at	Unknown	QLCU	NUC	Unknown	758.98	945.71	1,202.79	1,080.79
SRV_10451_at	Unknown	LU	NUC	Unknown	501.40	656.39	892.35	1,028.64
SRV_10498_at	Unknown	LU	NUC	Unknown	4,189.51	5,042.89	7,378.09	8,555.42
SRV_05470_at	Unknown	LU	UCC	Unknown	236.07	360.21	472.20	646.31
SRV_10359_at	Unknown	QLCU	UCC	Unknown	704.12	1,099.96	1,458.82	1,744.57
SRV_10554 s_at	Unknown	QLCU	UCC	Unknown	1,079.34	1,658.45	1,490.51	1,456.85

Probe-set ID represents the unique identifier for each probe-set on the custom *Ambystoma* GeneChip. The column "Pattern" describes the gene expression patterns on days 56, 70, and 84 post hatching and the column "Model" describes the regression models fit to the gene expression patterns on days 42, 56, 70, and 84 post hatching for genes uniquely expressed by ≥ 1.5 -fold in *A. mexicanum*. The columns denoted by "42 DPH", "56 DPH", "70 DPH", and "84 DPH" refer to the mean fold change observed on days 42, 56, 70 and 84 post hatching.

APPENDIX II
***A. t. tigrinum* significant genes**

Probe-set	Gene Symbol	Model	Pattern	Function	42 DPH	56 DPH	70 DPH	84 DPH
SRV_00116_at	A2M	LU	NNU	Protease inhibitor	310.71	309.83	337.10	480.37
SRV_00720_at	ABCF1	LD	NND	Stress/Immune response	673.92	534.74	475.36	420.39
SRV_11745_at	ACOX3	QLCU	NUC	Metabolism	596.53	844.25	982.31	1,063.81
SRV_00457_at	ADH1C	QLCU	NUC	Metabolism	453.52	600.41	738.23	576.31
SRV_00461_a_at	ADH4	LU	NNU	Unknown	276.26	341.94	394.95	494.25
SRV_00458_at	ADH5	QLCU	NUC	Metabolism	208.18	280.27	334.76	257.54
SRV_01095_a_at	AKR1B10	LD	NDC	Metabolism	2,007.98	1,556.52	1,278.82	998.01
SRV_00123_at	ALAD	LD	NDC	Metabolism	121.65	87.60	80.95	63.19
SRV_00124_a_at	ALAS2	LU	NUC	Stress/Immune response	847.86	958.73	1,458.51	1,445.92
SRV_00468_a_at	ALDH1A1	QLVU	NUU	Metabolism	363.08	418.05	598.13	1,232.39
SRV_02595_a_at	ALDH6A1	LD	NND	Metabolism	713.73	577.14	595.82	475.22
SRV_00752_a_at	ANG	LU	NNU	Cell differentiation	29.63	33.18	42.95	54.17
SRV_04283_at	ANLN	LD	NDD	Actin binding	112.78	93.00	69.90	45.14
SRV_04811_a_at	ANP32E	LD	NDC	Protein binding	5,176.80	4,198.79	3,407.49	2,841.05
SRV_00492_a_at	ANXA1	QLCU	UCC	Cell differentiation	846.82	1,325.47	1,394.66	1,165.17
SRV_00501_a_at	ANXA1_2	LU	NUU	Cell differentiation	67.17	89.34	130.18	270.78
SRV_00482_x_at	ANXA1_3	LU	NNU	Cell differentiation	986.38	1,040.68	1,389.64	1,486.65
SRV_05165_a_at	APEX1	QLCD	NND	Stress/Immune response	1,209.21	1,151.47	966.38	714.77
SRV_00130_a_at	APOE	LU	NUC	Stress/Immune response	3,379.26	3,016.68	5,245.28	6,559.79
SRV_00134_a_at	ASL	LU	NNU	Stress/Immune response	420.61	478.54	589.14	747.27
SRV_01139_a_at	ATP6V1C1	LU	NNU	Metabolism	2,603.02	2,993.96	3,776.01	4,570.32
SRV_01907_at	AURKA	QLCD	NDD	Cell cycle; M	969.94	800.35	528.27	260.62
SRV_02113_a_at	AURKB	LD	NDD	Cell cycle; M	707.70	543.35	400.08	249.03
SRV_02056_a_at	B2M	LU	NUU	Stress/Immune response	259.16	347.59	523.32	798.35
SRV_04888_a_at	B3GNT5	LD	NND	Central nervous system development	107.74	88.92	87.65	54.50
SRV_03405_at	BAZ1A	LD	NND	Transcription	551.47	465.44	380.04	341.73
SRV_04948_at	BAZ1B	LD	NND	Transcription	714.71	622.30	514.62	465.83

SRV_04737_at	C10ORF119	QLVD	NDC	DNA replication	664.51	477.82	365.53	308.49
SRV_10156_s_at	C10ORF54	QLVU	NNU	Receptor activity	106.84	114.36	131.69	191.99
SRV_05455_a_at	C11ORF31	LD	NND	Cell redox homeostasis	3,216.28	2,727.68	2,187.36	1,651.49
SRV_10084_at	C17ORF67	QLVD	DCC	Unknown	140.90	84.44	77.87	68.94
SRV_05942_at	C6ORF115	LD	NDC	Cell cycle; M	2,170.80	1,765.75	1,272.75	927.39
SRV_03504_a_at	CARHSP1	QLVD	NDC	Transcription: Regulation	1,410.46	1,069.74	939.73	805.47
SRV_02136_at	CARTPT	LU	NNU	Stress/Immune response	141.36	171.58	160.58	258.75
SRV_05022_at	CASP2	LD	NDC	Apoptosis	278.90	192.21	173.32	135.22
SRV_01167_a_at	CAT	LU	UCU	Stress/Immune response	385.55	916.60	1,265.16	2,877.39
SRV_00799_at	CCNA2	QLCD	NDD	Cell cycle: Checkpoint	392.80	298.96	192.91	109.96
SRV_04877_a_at	CCNB1	QLCD	NDD	Cell cycle; G2/M	550.70	426.42	267.79	154.42
SRV_05031_at	CCNB3	LD	NDD	Cell cycle	456.68	355.54	217.45	123.22
SRV_02062_at	CCNG1	QLVU	NNU	Cell cycle	4,547.70	4,948.08	5,896.53	7,468.84
SRV_02274_at	CD74	LU	NNU	Stress/Immune response	260.69	297.67	388.04	446.54
SRV_01173_at	CD9	LU	NUC	Signal transduction	718.68	1,064.71	1,358.67	1,628.29
SRV_01180_at	CDC2	QLCD	NDD	Cell cycle	1,139.52	906.99	629.33	304.68
SRV_00806_a_at	CDC20	QLCD	NDD	Cell cycle; M	796.99	620.64	407.66	246.53
SRV_01183_at	CDC25A	LD	NDC	Cell cycle; G1/S	214.92	184.98	139.26	104.53
SRV_11238_at	CDC25C	LD	NDC	Cell cycle	92.28	67.81	55.42	42.31
SRV_00804_at	CDC6	LD	NDC	Cell cycle; DNA replication	264.12	180.70	151.61	101.73
SRV_11747_at	CDC7	LD	NND	Cell cycle	229.21	166.30	181.52	149.65
SRV_04156_at	CDCA8	QLCD	NDD	Cell cycle; M	416.70	406.03	255.12	156.12
SRV_00319_at	CDH1	LU	NNU	Cell-cell adhesion	165.47	186.89	232.48	274.35
SRV_11243_at	CDK2	LD	NND	Cell cycle; G2/M	309.12	268.21	223.93	173.96
SRV_07007_a_at	CDK4	LD	NND	Cell cycle; G1/S	152.90	108.04	107.80	91.22
SRV_02063_at	CDKN1B	QLVU	NUU	Cell cycle; G1/S	235.33	182.46	274.46	725.97
SRV_05168_at	CDKN2AIPNL	QLVD	NND	Unknown	2,876.81	2,266.70	2,000.53	1,791.64
SRV_02151_a_at	CETN2	LD	NND	Cell cycle; M	1,070.05	944.49	764.01	683.11
SRV_02551_at	CHAF1A	LD	NND	Response to DNA damage stimulus	214.99	180.05	150.97	122.96
SRV_00813_at	CHD4	LD	NDC	DNA metabolism	1,729.35	1,456.48	1,024.04	899.31
SRV_00815_a_at	CHEK1	LD	NDC	Cell cycle; DNA damage	180.32	128.74	110.77	84.88
SRV_04406_at	CHPT1	LU	NNU	Lipid metabolism	255.95	322.54	344.28	384.84
SRV_01199_a_at	CLU	LU	UCC	Cell differentiation	901.42	1,390.53	1,571.30	2,054.97

SRV_04978_a_at	CNDP1	LU	NNU	Peptidase activity	224.92	219.32	317.51	345.06
SRV_04977_at	CNDP2	LU	NNU	Dipeptidase activity	5,793.43	6,930.44	8,371.70	10,494.37
SRV_03476_at	CNPY2	LU	NNU	Protein binding	262.89	339.42	323.93	407.16
SRV_05158_a_at	COL11A1	LD	NDC	Cell adhesion	422.04	323.32	244.93	199.10
SRV_02163_at	COL12A1	LU	NNU	Cell adhesion	199.07	234.71	260.23	347.65
SRV_09970_at	COL1A2	LD	NND	ECM component	835.40	729.57	731.29	505.75
SRV_01205_at	COL2A1	QLVD	DCC	Organ development	747.89	458.11	337.71	279.02
SRV_10560_a_at	COL3A1	LD	NND	ECM component	2,904.94	2,531.12	2,290.36	1,536.25
SRV_11255_at	COL4A1	LD	NND	ECM component	636.44	599.44	535.95	401.38
SRV_11256_at	COL4A2	LD	NND	ECM component	623.08	589.88	551.21	406.52
SRV_01225_at	CRYAB	LU	NUC	Signal transduction	125.63	176.64	201.93	243.25
SRV_00149_a_at	CST3	LU	NUC	Tissue remodeling	1,880.81	2,484.94	3,313.77	4,645.88
SRV_00326_a_at	CTSK	LU	NNU	Proteolysis	630.81	669.22	857.46	1,144.25
SRV_01877_at	CXCR4	LD	NND	Stress/Immune response	583.64	459.79	408.98	360.61
SRV_00515_at	CYP27C1	QLCD	NND	Metal ion binding	555.87	525.14	595.97	358.57
SRV_10891_at	CYP2A13	LU	NNU	Oxidation reduction	53.61	68.92	76.78	104.85
SRV_10570_at	CYP2D6	LU	NUC	Oxidation reduction	90.42	105.62	145.99	193.15
SRV_02179_a_at	DAP	LU	NNU	Apoptosis	1,951.07	2,100.95	2,598.96	3,079.33
SRV_03593_at	DLG7	QLCD	NND	Cell cycle; M	205.38	185.32	150.08	93.76
SRV_03680_at	DNAJC9	LD	NND	Heat shock protein binding	391.42	321.42	290.58	242.41
SRV_00888_at	DNMT1	LD	NND	Transcription	1,194.51	1,005.16	851.80	728.53
SRV_02777_at	DSCR3	LU	NNU	Vacuolar transport	917.59	1,020.29	1,213.29	1,400.45
SRV_01258_at	DUT	LD	NND	Metabolism	3,616.48	2,991.68	2,447.65	1,889.48
SRV_02192_at	DYNC1H1	LU	NUC	Protein binding	408.62	609.96	692.36	718.54
SRV_07255_at	ESCO2	LD	NDD	Cell cycle; S	269.56	211.91	170.17	110.83
SRV_03489_a_at	EXOSC2	LD	NDC	rRNA processing	571.54	468.90	371.81	351.48
SRV_05260_a_at	F11R	LU	NNU	Cell adhesion	91.68	125.62	131.87	148.55
SRV_00949_at	FAAH	QLVU	NNU	Fatty acid hydrolysis	189.37	218.57	261.02	407.75
SRV_00160_s_at	FAH	LD	NND	Metabolism	663.78	558.45	486.64	410.33
SRV_05626_at	FAM79A	LU	NNU	Synaptic vesicle component	88.03	82.55	96.33	166.98
SRV_01278_at	FBN2	LD	NND	Extracellular matrix structural constituent	122.82	94.49	82.32	68.67
SRV_02084_at	FEN1	LD	NDC	Stress/Immune response	864.98	726.60	564.82	408.44
SRV_02451_at	FGFBP1	QLCU	UCC	FGF signaling	496.40	755.42	970.38	982.73

SRV_00953_a_at	FHL1	LU	NNU	Cell differentiation	178.18	188.11	258.25	291.74
SRV_02203_a_at	FKBP2	QLVU	NNU	Protein binding	2,127.25	2,667.22	2,888.75	4,495.08
SRV_01287_at	FRK	LU	NNU	Cell cycle	70.17	89.82	97.45	108.89
SRV_00167_a_at	FTH1	LU	UUU	Iron homeostasis	1,325.76	2,778.50	5,217.13	8,862.29
SRV_01290_at	FTH1_2	LD	NND	Iron homeostasis	1,368.65	1,120.26	1,051.02	825.03
SRV_02740_a_at	FXYD3	LU	NNU	Ion transport	2,142.68	2,585.48	2,947.36	3,302.38
SRV_01304_at	GFAP	QLVD	NDC	Protein binding	6,454.24	4,709.13	4,049.26	3,512.69
SRV_09706_at	GLUL	QLVU	NUC	Metabolism	4,885.01	5,696.74	7,587.71	10,552.89
SRV_02362_at	GMFG	LU	NNU	Actin binding	357.21	493.86	506.34	557.35
SRV_01466_at	GPNMB	QLVU	NNU	Cell differentiation	78.43	97.35	129.11	622.61
SRV_00444_a_at	GPX1	LU	NUC	Stress/Immune response	1,212.60	1,488.58	2,346.69	3,470.79
SRV_02502_a_at	H3F3B	LD	NDC	DNA metabolism	3,604.52	2,870.58	1,990.02	1,553.90
SRV_00424_x_at	HBA2_1	QLCD	NDD	Oxygen transport	20,773.07	14,085.87	6,712.25	413.95
SRV_00397_s_at	HBA2_2	QLCU	UCC	Oxygen transport	18,579.01	32,024.22	33,390.71	32,945.06
SRV_02509_copy2_at	HBA2_3	QLVD	DCC	Oxygen transport	726.64	137.39	105.05	78.73
SRV_00375_s_at	HBD	QLCU	UCC	Oxygen transport	17,980.22	27,039.92	29,125.87	28,890.15
SRV_00186_a_at	HBE1	QLCD	NDD	Oxygen transport	30,583.41	24,339.08	15,222.07	1,626.03
SRV_00434_x_at	HBG1	QLCU	UCC	Oxygen transport	18,702.13	28,153.58	30,441.18	30,683.60
SRV_00037_at	HBZ	LD	DDD	Oxygen transport	9,796.87	1,749.97	235.27	63.79
SRV_00387_at	HEXA	LU	NNU	Metabolism	755.39	910.00	1,068.64	1,400.65
SRV_02500_at	HIST1H1B	LD	NDC	DNA binding	315.34	213.52	192.05	148.38
SRV_04494_at	HIST1H2BJ	QLCD	NDD	Defense response	20,442.96	17,160.00	13,031.91	6,886.06
SRV_05541_at	HIST2H2AC	LD	NDD	DNA binding	465.39	328.40	251.13	152.11
SRV_08158_s_at	HMGB2	QLCD	NND	Transcription factor	15,136.49	13,781.01	11,691.83	9,696.98
SRV_04827_a_at	HNRNP A2B1	QC	UDC	RNA binding	142.09	352.58	234.13	214.63
SRV_00992_x_at	HNRNPL	QLCD	NND	RNA binding	10,314.63	10,382.18	8,285.82	6,852.47
SRV_02219_a_at	HNRNPU	LD	NDC	RNA splicing	2,667.09	2,196.84	1,711.99	1,550.73
SRV_04356_at	HNRPH2	QLCD	NND	RNA binding	9,759.36	9,648.90	7,683.16	5,952.23
SRV_01329_at	HSD17B2	LD	NND	Response to retinoic acid	829.75	716.32	563.81	340.31
AH_at	HSPA8	QLVD	NDC	Stress/Immune response	5,359.16	3,626.04	3,362.90	2,978.03
SRV_02989_at	HSPA8_1	LU	NUC	Stress/Immune response	351.77	461.79	543.57	732.56
SRV_02573_a_at	IDH3A	LU	NNU	Metabolism	1,458.44	1,621.13	1,963.37	2,252.07
SRV_03329_at	IFIT5	QLVU	NNU	Binding	26.01	27.18	36.48	48.97

SRV_04367_a_at	IGLL1	LU	NUU	Stress/Immune response	52.47	57.92	101.70	156.74
SRV_07449_x_at	ILF3	LD	NDC	Cell cycle	3,645.63	2,892.97	2,313.63	2,059.73
SRV_04145_a_at	IPO9	LD	NND	Nuclear transport	647.45	563.89	443.93	370.62
SRV_02442_a_at	ISG15	LU	NUC	Cell-cell signaling	50.36	54.69	78.82	93.12
SRV_05450_a_at	ITGB1BP3	QLVU	NNU	Metabolism	1,465.52	1,187.51	1,233.95	2,382.57
SRV_01346_a_at	IVD	LD	NND	Oxidation reduction	786.65	697.30	634.28	505.34
SRV_02395_a_at	JARID2	QLVU	DUC	Central nervous system development	874.56	462.45	875.15	1,174.10
SRV_01942_a_at	KHSRP	QLVD	NDC	Transcription	2,466.07	1,912.96	1,502.28	1,333.80
SRV_03588_at	KIAA0101	QLCD	NDD	Cell cycle; S	588.38	418.53	294.97	159.39
SRV_02235_at	KIF11	QLCD	NDD	Cell cycle; M	364.38	322.27	221.68	109.10
SRV_03220_at	KIF22	LD	NDC	Nuclear transport	185.99	169.06	116.55	111.28
SRV_02578_at	KIFC1	LD	NDD	Cell cycle; M	703.47	537.93	413.71	258.14
SRV_01355_at	KPNA2	QLCD	NND	Intracellular protein transport	4,505.35	4,011.12	3,125.30	2,013.86
SRV_01353_a_at	KPNB1	LD	NND	Nuclear transport	1,741.34	1,469.52	1,335.25	1,123.95
SRV_10623_x_at	KRT12	QLVD	DDC	Cytoskeleton	360.91	103.22	61.96	44.39
SRV_02580_a_at	LAMA1	LD	NDC	ECM component	115.84	91.83	75.23	55.53
SRV_02581_a_at	LAMP1	LU	NNU	Lysosomal exocytosis	438.62	586.76	630.66	708.28
SRV_01387_at	LGALS1	LU	NUC	Cell differentiation	998.20	1,385.01	1,722.59	2,503.93
SRV_00260_at	LIG1	LD	NDC	Response to DNA damage stimulus	236.53	213.17	155.51	137.47
SRV_02591_at	LMNB1	LD	NND	Nuclear structural molecule	477.05	432.17	359.55	272.59
SRV_01392_at	LMX1B	LD	NND	Transcription factor	149.81	124.48	112.52	93.61
SRV_00520_at	LOC643509	LD	NDC	Unknown	752.68	587.42	484.15	374.85
SRV_07838_a_at	LOH1ICR2A	LU	NNU	Cell cycle	332.89	367.52	485.00	500.25
SRV_03274_a_at	LTB4DH	LU	NNU	Metabolism	150.97	187.03	193.73	235.59
SRV_01409_at	MAD2L1	LD	NDD	Cell cycle; M	414.80	291.62	211.88	125.82
SRV_03054_at	MARCO	QLVU	NNU	Signal transduction	50.48	62.09	70.93	147.72
SRV_01414_at	MCM3	QLCD	NDD	Cell cycle; DNA replication	615.38	436.21	311.17	168.27
SRV_05623_at	MCM4	QLCD	NDD	DNA metabolism	774.23	548.15	326.49	168.85
SRV_02725_a_at	MCM6	QLCD	NDD	Cell cycle; S	891.85	693.21	489.58	292.90
SRV_02727_at	MCM7	LD	NDD	Cell cycle; DNA replication	1,484.93	1,028.67	703.30	427.05
SRV_00081_at	MED24	LD	NND	Transcription/Thyroid hormone receptor binding	264.18	226.28	197.95	165.39
SRV_04611_a_at	MLF1	LU	NNU	Cell differentiation	592.69	852.85	784.89	953.34
SRV_00060_s_at	MR1	LU	NUC	Stress/Immune response	877.34	1,229.50	1,435.25	1,648.67

SRV_00011_at	MSX1	LD	NDC	Transcription	54.03	46.29	34.18	33.68
SRV_03007_a_at	MTHFD2	LU	NUC	Metabolism	1,461.35	1,763.74	2,209.62	2,700.81
SRV_01439_a_at	MX1	LU	NNU	Defense response	28.84	39.43	37.23	65.33
SRV_03882_at	MYEF2	LD	NND	Transcription	3,698.11	3,469.48	2,734.34	2,360.48
SRV_12418_at	MYL9	QLVD	NDC	Biological process	458.74	344.37	282.69	277.88
SRV_02522_a_at	MYO1B	LU	NNU	Actin binding	115.93	127.42	150.50	189.97
SRV_01990_a_at	NAPG	QLVU	NNU	Intracellular protein transport	854.43	838.56	1,093.41	1,327.33
SRV_05464_at	NASP	LD	NND	DNA replication	3,546.35	2,981.57	2,524.36	2,339.52
SRV_07976_a_at	NCOA5	LD	NND	Transcription	3,718.98	2,909.30	2,645.20	2,298.86
SRV_04199_at	NEIL3	LD	NND	Response to DNA damage stimulus	115.78	91.82	83.29	74.26
SRV_14350_at	NEK3	QLCU	UCC	Cell cycle; M	201.82	320.42	301.64	266.60
SRV_02410_a_at	NHP2L1	LD	NND	RNA binding	2,870.22	2,374.22	2,061.99	1,822.51
SRV_03227_at	NID2	LD	NND	Cell adhesion	702.17	628.45	577.26	427.80
SRV_04153_a_at	NLE1	QLVD	NND	Unknown	116.43	88.43	79.17	77.50
SRV_02904_at	NOL5A	QLVD	NDC	RNA binding	1,487.13	1,201.24	941.82	942.26
SRV_00080_at	NPY	LU	NNU	Stimulus response	1,448.20	1,658.01	1,971.12	2,193.93
SRV_00540_a_at	NR3C2	LU	NUU	Transcription factor	110.91	143.99	197.01	306.81
SRV_04423_a_at	NUP107	LD	NND	Intracellular protein transport	280.22	230.30	190.09	152.77
SRV_04713_a_at	NUP43	LD	NND	Intracellular protein transport	430.97	375.65	317.82	280.38
SRV_10040_a_at	NUP50	QLVD	NDC	Intracellular protein transport	141.68	105.32	87.17	94.09
SRV_03323_a_at	NUPR1	LU	NNU	Signal transduction	532.09	705.29	783.82	814.85
SRV_04253_a_at	NUSAP1	QLCD	NND	Cell cycle; M	736.78	718.54	552.48	364.68
SRV_02098_at	ORC1L	LD	NDC	DNA replication	132.76	91.74	85.39	66.80
SRV_01488_at	PAK1	LU	NNU	Signal transduction	575.02	734.65	806.71	910.89
SRV_01092_at	PARP1	LD	NND	Stress/Immune response	543.34	460.27	368.74	288.74
SRV_04270_at	PBK	QLCD	NND	Cell cycle; M	646.93	628.05	472.99	317.95
SRV_05605_a_at	PCNA	LD	NDC	Cell cycle	6,174.40	4,869.31	3,616.57	2,476.44
SRV_03382_a_at	PCOLCE2	LU	NNU	Protein binding	119.74	121.11	159.74	206.11
SRV_03540_at	PDCD4	QLVU	NNU	Apoptosis	804.13	856.64	1,100.90	1,332.50
SRV_01495_at	PDK2	LU	NNU	Signal transduction	794.79	940.52	1,070.11	1,325.34
SRV_12805_at	PFAS	LD	NDC	Metabolism	106.45	83.38	69.47	65.80
SRV_01945_a_at	PLA2G4C	LU	NUC	Stress/Immune response	40.46	47.83	65.68	61.30
SRV_04120_at	PLEKHB2	LU	NNU	Protein binding	68.38	73.54	91.74	124.98

SRV_02420_at	PLK1	QLCD	NDD	Cell cycle; M	891.56	622.25	408.11	194.38
SRV_04514_at	POLR2L	QLVU	NUU	Transcription	147.70	122.69	202.96	386.47
SRV_03782_at	POP5	LD	NDC	Metabolism	1,356.01	1,230.42	788.17	601.71
SRV_02945_at	POSTN	LD	NDD	Cell adhesion	361.29	247.47	127.25	65.52
SRV_02842_at	PPARD	LD	DDC	Transcription/Steroid hormone receptor	482.24	303.09	197.79	230.00
SRV_01534_at	PPP1CA	LD	NND	Cell cycle	2,315.27	1,912.86	1,594.51	1,444.00
SRV_00559_a_at	PRIMI	LD	NDC	DNA replication	780.22	612.15	501.83	341.44
SRV_00560_at	PRL	LU	NNU	Pituitary hormone	82.49	91.27	108.55	136.94
SRV_01558_at	PSAP	LD	DCC	Metabolism	191.68	120.97	124.58	91.62
SRV_04881_at	PTBP1	LD	NDC	Metabolism	776.72	721.78	504.67	401.22
SRV_00562_a_at	PTGS1	LD	NDC	Stress/Immune response	276.33	203.74	159.35	125.46
SRV_01617_a_at	PTX3	LD	NND	Stress/Immune response	247.93	211.45	187.09	148.08
SRV_02282_a_at	RAB7A	LU	NNU	Signal transduction	290.84	366.81	409.07	501.73
SRV_01638_at	RANBP1	QLVD	NND	Signal transduction	3,644.16	2,864.49	2,473.97	2,193.41
SRV_04387_at	RARRES1	LU	NUC	Negative regulation of cell proliferation	2,652.51	3,484.21	3,993.84	4,836.23
SRV_04184_at	RAVER2	LD	NDC	RNA binding	422.58	326.37	273.11	236.52
AN_s_at	RBMX	QLCD	NND	RNA binding	9,359.92	9,254.60	7,250.96	6,054.75
SRV_00812_a_at	RCC1	QLVD	DCC	Cell cycle; M	595.06	390.59	319.56	267.12
SRV_04287_at	RCC2	LD	NND	Cell cycle; M	214.10	185.48	156.20	117.68
SRV_05574_at	RFC2	LD	NDC	DNA replication	761.93	659.76	501.79	397.41
SRV_05578_at	RFC4	LD	NND	DNA replication	252.03	208.46	197.68	163.00
SRV_02852_a_at	RGPD2	LD	NDC	Intracellular transport	137.03	104.63	90.36	84.10
SRV_05270_a_at	RHEBL1	LD	NDC	Signal transduction	75.78	55.20	49.92	45.93
SRV_01991_a_at	RIOK3	LU	NNU	Protein binding	1,057.23	1,210.98	1,547.43	2,017.33
SRV_02906_a_at	RNASEH2A	LD	NDC	DNA replication	698.98	550.21	458.98	384.17
SRV_04699_at	RNASEH2B	LD	NND	DNA replication	1,331.55	1,015.22	1,019.63	825.20
SRV_03601_at	RNF40	QLVD	NDC	Ion binding	224.54	156.50	135.62	140.76
SRV_11572_a_at	RPA2	LD	NDC	DNA replication	966.79	743.59	571.16	489.27
SRV_00053_at	RPE65	LU	NNU	Stimulus response	412.20	549.74	592.97	742.41
SRV_00638_at	RPL31	LD	NND	Protein synthesis	163.49	120.87	109.62	97.34
SRV_02269_a_at	RRBP1	LU	NNU	Signal transduction	219.46	249.22	277.97	333.00
SRV_00705_at	RRM1	QLCD	NDD	Cell cycle; DNA replication	1,452.13	1,160.86	795.97	501.55
SRV_00706_a_at	RRM2	LD	DDD	Cell cycle; DNA replication	1,472.71	936.70	607.32	299.70

SRV_03157_at	RSL1D1	QLVD	NDC	RNA processing	899.69	690.40	580.48	586.90
SRV_02216_a_at	S100A2	LD	DDC	Ion binding	704.06	428.71	197.81	165.16
SRV_01675_s_at	SAFB	QLCD	NND	Transcription	2,451.31	2,342.19	1,981.72	1,611.48
SRV_08093_at	SEPI1	LU	NNU	Stress/Immune response	5,527.38	6,766.97	8,276.40	11,001.87
SRV_04195_at	SEPT8	LU	NUC	Cell cycle	500.02	575.11	802.82	892.77
SRV_04787_s_at	SERPINB2	LU	NUC	Anti-apoptosis	186.42	211.17	288.12	300.14
SRV_00796_at	SERPINH1	QC	UDC	Stress/Immune response	99.41	193.23	124.34	89.06
SRV_03108_a_at	SFRS1	QLCD	NND	RNA binding	6,222.55	6,289.35	4,590.22	3,423.92
SRV_05466_at	SFRS14	LD	NND	RNA binding	6,384.34	5,473.36	4,581.56	3,499.10
SRV_02105_a_at	SHMT1	QLVD	DCC	Metabolism	831.36	550.84	465.27	390.12
SRV_02964_s_at	SLBP	LD	NDC	mRNA processing	609.94	477.36	380.35	303.63
SRV_01681_a_at	SLTM	LD	NND	Transcription	930.31	799.34	742.10	605.30
SRV_01909_at	SMARCA5	LD	NDC	Transcription	1,946.81	1,584.63	1,286.17	1,008.27
SRV_02556_at	SMC4	QLCD	NDD	Cell cycle; M	492.58	470.13	314.49	177.90
SRV_01716_a_at	SNRP70	QLVD	NND	RNA binding	2,642.53	2,099.30	1,803.54	1,743.82
SRV_02613_at	SOX3	LD	NDC	Transcription factor	535.71	468.05	333.03	310.49
SRV_03652_at	SPEN	QLVD	NDC	Transcription	2,014.99	1,372.50	1,284.34	1,169.11
SRV_00712_at	SSTR5	LD	NND	Somatostatin receptor activity	178.94	151.81	146.59	97.03
SRV_01758_at	STXBP3	LU	NNU	Protein transport	668.48	691.83	883.38	1,034.78
SRV_03675_at	SULF1	LD	NND	Apoptosis	307.07	247.82	231.17	178.29
SRV_01767_at	TAGLN	QLVU	NNU	Actin binding	102.38	108.78	122.77	226.64
SRV_01775_at	TEAD4	LD	NDC	Transcription	68.10	51.22	41.92	37.35
SRV_03161_a_at	TFDP1	LD	NND	Cell cycle	322.63	302.74	250.02	212.13
SRV_04265_a_at	THEM2	LU	NNU	Hydrolase activity	337.46	401.78	441.26	507.82
SRV_04205_at	THNSL2	QLCU	NUC	Metabolism	49.10	62.20	74.88	70.92
SRV_01791_at	TIAM1	LU	NNU	Signal transduction	592.83	667.66	884.00	1,189.25
SRV_03341_at	TIMM13	QLVD	NDC	Intracellular protein transport	1,567.91	1,090.85	923.14	900.61
SRV_11663_a_at	TIMP1	LU	NUC	Cell maturation	160.13	238.23	241.87	321.27
SRV_04894_a_at	TKTL2	LD	NND	Metabolism	7,163.45	5,831.98	5,560.86	4,737.20
SRV_04650_a_at	TM6SF1	LU	NNU	Unknown	160.89	160.26	234.31	304.09
SRV_05017_at	TMEM128	LU	NNU	Unknown	525.00	632.48	741.06	849.24
SRV_03419_at	TMEM176B	LU	NUC	Organ morphogenesis	897.54	1,276.69	1,639.11	2,081.10
SRV_01795_at	TMPO	LD	NDC	Transcription	914.33	769.70	574.18	453.33

SRV_03257_at	TPX2	QLCD	NDD	Cell proliferation	1,368.64	1,220.81	842.96	418.49
SRV_00718_at	TYMS	QLCD	NND	Phosphoinositide-mediated signaling	502.10	441.07	377.55	277.56
SRV_01821_at	TYROBP	LU	NNU	Stress/Immune response	69.79	74.76	84.41	120.13
SRV_03215_a_at	U2AF2	QLVD	NND	RNA binding	5,919.00	4,561.06	4,256.45	3,886.00
SRV_01735_a_at	UAP1L1	LU	NNU	Metabolism	212.75	221.53	271.12	358.98
SRV_03361_at	UHRF1	LD	DDD	Cell cycle	598.88	398.08	263.17	151.96
SRV_03360_at	UHRF2	LD	NDD	Cell cycle: Regulation	849.93	612.11	365.06	197.97
SRV_00518_a_at	Unknown	QLCD	NND	Unknown	87.43	79.45	88.34	54.38
SRV_02170_a_at	Unknown	LU	NNU	Unknown	140.02	181.73	199.28	225.40
SRV_02860_at	Unknown	LD	NND	Unknown	3,844.75	3,029.80	2,760.77	2,433.64
SRV_03653_at	Unknown	QLVD	NND	Unknown	2,077.31	1,602.59	1,439.81	1,370.77
SRV_04604_s_at	Unknown	LU	NUC	Unknown	88.49	102.73	143.44	148.82
SRV_05024_at	Unknown	LD	DCC	Unknown	190.18	113.23	107.16	73.88
SRV_05381_at	Unknown	QLVD	NND	Unknown	824.46	685.84	561.35	541.93
SRV_05671_at	Unknown	QLCD	NND	Unknown	1,329.14	1,259.44	1,077.30	764.78
SRV_05676_at	Unknown	QLCU	UCC	Unknown	947.02	1,796.01	1,989.56	1,953.73
SRV_05709_at	Unknown	LU	NNU	Unknown	4,335.64	5,605.63	5,896.35	8,454.46
SRV_05710_a_at	Unknown	LU	NNU	Unknown	7,986.86	9,288.74	9,837.38	12,139.43
SRV_05730_a_at	Unknown	LU	NNU	Unknown	98.23	127.55	128.65	169.41
SRV_05931_a_at	Unknown	QLVU	NNU	Unknown	86.91	80.66	89.30	174.11
SRV_06044_a_at	Unknown	LD	NND	Unknown	372.07	292.45	257.56	243.24
SRV_06116_at	Unknown	LU	NUC	Unknown	51.50	51.19	81.60	86.25
SRV_06267_at	Unknown	QLCD	NND	Unknown	4,005.35	3,812.27	2,942.12	2,010.60
SRV_06453_at	Unknown	LU	NNU	Unknown	1,822.78	2,132.64	2,558.86	2,962.20
SRV_06479_a_at	Unknown	LU	NUC	Unknown	1,873.90	2,511.40	2,936.83	3,604.04
SRV_06526_a_at	Unknown	LU	NNU	Unknown	485.53	566.14	625.73	792.65
SRV_06778_at	Unknown	LD	NDC	Unknown	222.56	160.37	130.57	96.95
SRV_06910_a_at	Unknown	LD	NND	Unknown	3,201.71	2,857.41	2,450.50	2,060.80
SRV_07143_at	Unknown	LU	NNU	Unknown	1,642.59	1,881.71	2,139.22	2,515.18
SRV_07147_s_at	Unknown	QLCU	NUC	Unknown	249.10	333.01	397.39	455.18
SRV_07227_at	Unknown	QLCD	NND	Unknown	193.43	235.04	172.77	118.07
SRV_07258_at	Unknown	LU	NUC	Unknown	1,642.07	2,000.11	2,630.76	2,628.39
SRV_07312_at	Unknown	QLVU	DUC	Unknown	841.52	528.24	898.41	1,133.45

SRV_07418_at	Unknown	LD	NND	Unknown	2,405.82	2,180.64	1,644.34	1,420.57
SRV_07467_at	Unknown	LD	NDC	Unknown	1,752.67	1,454.07	1,158.98	952.10
SRV_07537_at	Unknown	QLVU	NNU	Unknown	192.21	205.06	231.53	290.68
SRV_07538_at	Unknown	LU	NUU	Unknown	1,589.53	2,125.70	2,729.10	4,771.02
SRV_07581_at	Unknown	LU	NNU	Unknown	56.73	56.80	71.35	88.77
SRV_07583_at	Unknown	LD	NND	Unknown	5,638.47	4,964.30	3,940.59	3,283.44
SRV_07610_at	Unknown	LU	NUC	Unknown	4,909.57	5,453.69	7,885.78	10,883.67
SRV_07632_at	Unknown	LD	DCD	Unknown	354.76	121.13	122.19	66.98
SRV_07640_a_at	Unknown	LD	NND	Unknown	6,886.95	5,156.84	4,727.12	3,936.96
SRV_07663_at	Unknown	LD	NND	Unknown	1,661.41	1,368.89	1,167.31	889.13
SRV_07680_a_at	Unknown	LU	NNU	Unknown	27.52	31.76	36.31	42.16
SRV_07693_at	Unknown	LU	NNU	Unknown	1,141.62	1,308.72	1,635.76	1,758.54
SRV_07699_at	Unknown	LU	NNU	Unknown	67.84	78.60	96.18	125.28
SRV_07724_at	Unknown	LU	NNU	Unknown	172.15	224.84	253.52	297.55
SRV_07728_a_at	Unknown	LD	NND	Unknown	648.12	532.53	444.41	357.86
SRV_07734_at	Unknown	QLVU	NNU	Unknown	61.29	70.95	76.94	118.23
SRV_07780_at	Unknown	LU	NUC	Unknown	518.09	597.50	814.79	1,020.80
SRV_07819_at	Unknown	LU	NUC	Unknown	165.81	197.80	257.52	279.54
SRV_07842_s_at	Unknown	LU	UUU	Unknown	210.66	538.46	1,117.27	2,415.53
SRV_07927_at	Unknown	LU	NNU	Unknown	680.31	777.46	900.43	1,054.26
SRV_08071_a_at	Unknown	LU	NNU	Unknown	25.35	27.10	30.87	39.57
SRV_08278_at	Unknown	LU	NNU	Unknown	467.22	555.53	674.04	716.76
SRV_08311_at	Unknown	LD	NDC	Unknown	1,813.84	1,297.25	1,082.20	940.90
SRV_08867_at	Unknown	QLCD	NND	Unknown	976.46	961.14	802.26	606.22
SRV_09284_at	Unknown	LU	NNU	Unknown	688.06	850.24	947.63	1,122.30
SRV_09392_at	Unknown	LU	NUC	Unknown	52.35	69.65	93.72	130.22
SRV_09401_at	Unknown	LD	NND	Unknown	749.57	652.75	543.86	443.25
SRV_09457_at	Unknown	LD	NND	Unknown	434.05	367.47	322.36	208.70
SRV_09490_at	Unknown	QLCU	UCC	Unknown	378.13	670.03	839.46	1,118.51
SRV_09512_at	Unknown	LU	NNU	Unknown	1,168.91	1,267.62	1,665.42	1,978.97
SRV_09536_at	Unknown	QLCU	UCC	Unknown	174.80	286.45	293.43	296.63
SRV_09568_at	Unknown	LU	NNU	Unknown	448.05	627.95	636.61	884.96
SRV_09588_s_at	Unknown	LU	NUC	Unknown	327.85	444.45	515.48	564.27

SRV_09700_at	Unknown	QC	UCC	Unknown	770.93	1,801.88	1,209.60	1,042.40
SRV_09703_at	Unknown	LU	NNU	Unknown	219.44	269.50	291.59	373.67
SRV_09921_at	Unknown	LD	NND	Unknown	3,174.42	2,567.94	2,392.78	2,043.19
SRV_09933_at	Unknown	LU	NNU	Unknown	808.08	865.74	1,070.30	1,215.89
SRV_10019_at	Unknown	LU	NUC	Unknown	3,665.72	4,705.78	6,726.31	8,961.52
SRV_10030_a_at	Unknown	LU	NNU	Unknown	292.72	332.68	394.13	440.20
SRV_10047_at	Unknown	LU	NNU	Unknown	118.04	133.20	167.14	210.30
SRV_10060_at	Unknown	LU	UCC	Unknown	170.66	292.82	289.09	394.96
SRV_10064_at	Unknown	QLVU	NNU	Unknown	25.69	32.01	30.70	91.28
SRV_10078_at	Unknown	LD	NND	Unknown	7,478.27	6,776.18	5,624.83	4,574.04
SRV_10114_at	Unknown	LU	NNU	Unknown	167.21	195.42	240.68	351.40
SRV_10146_at	Unknown	LD	NDC	Unknown	324.34	216.34	198.93	152.58
SRV_10183_at	Unknown	LD	NND	Unknown	4,632.49	3,933.69	3,512.40	3,035.28
SRV_10257_s_at	Unknown	QLVU	NNU	Unknown	147.06	150.81	181.68	243.99
SRV_10268_at	Unknown	LD	NND	Unknown	954.04	807.45	638.62	591.77
SRV_10284_at	Unknown	LU	NNU	Unknown	1,866.41	2,060.68	2,779.74	3,261.80
SRV_10291_at	Unknown	LU	NNU	Unknown	869.00	938.76	1,046.31	1,352.14
SRV_10292_at	Unknown	LD	NDC	Unknown	1,449.89	1,243.73	924.38	936.02
SRV_10305_at	Unknown	LU	NUC	Unknown	570.09	698.30	862.07	1,002.97
SRV_10312_at	Unknown	QLCU	UCC	Unknown	391.68	589.41	588.61	566.24
SRV_10321_at	Unknown	LU	NNU	Unknown	3,285.06	4,060.33	4,675.55	5,175.43
SRV_10323_at	Unknown	LD	NND	Unknown	3,986.19	3,612.33	2,875.62	2,401.96
SRV_10327_at	Unknown	LU	NNU	Unknown	651.27	839.26	873.48	1,131.96
SRV_10367_at	Unknown	LU	NNU	Unknown	2,005.49	2,374.22	2,845.19	3,566.08
SRV_10464_s_at	Unknown	LD	NDC	Unknown	5,164.03	5,332.69	3,543.61	3,250.42
SRV_01943_a_at	Unknown	LD	NND	Unknown	1,286.28	1,149.89	881.10	817.30
SRV_02137_at	Unknown	LU	NNU	Unknown	145.32	192.96	213.31	317.12
SRV_05470_at	Unknown	QLVU	NNU	Unknown	98.62	100.37	127.98	166.97
SRV_05721_at	Unknown	LD	NDC	Unknown	1,877.25	1,545.11	1,233.70	967.61
SRV_05804_at	Unknown	QLVD	DCC	Unknown	221.00	76.67	57.42	42.30
SRV_06364_a_at	Unknown	LD	NDC	Unknown	422.15	350.42	267.85	199.71
SRV_06485_a_at	Unknown	QLCD	NDD	Unknown	232.99	195.50	144.43	94.96
SRV_06667_a_at	Unknown	LD	NND	Unknown	984.23	858.27	713.73	620.47

SRV_06712_a_at	Unknown	LU	NNU	Unknown	6,506.04	7,313.73	9,122.99	9,828.00
SRV_06956_at	Unknown	LD	NDC	Unknown	1,598.68	1,313.59	925.77	622.16
SRV_06965_at	Unknown	LD	NND	Unknown	515.01	441.46	353.30	271.78
SRV_07004_at	Unknown	LD	NDD	Unknown	760.57	583.64	384.83	236.71
SRV_07072_x_at	Unknown	LU	NUC	Unknown	2,357.77	2,941.50	3,797.71	5,448.34
SRV_07203_at	Unknown	LD	NND	Unknown	187.43	155.07	131.43	110.36
SRV_07269_at	Unknown	QLCD	NDD	Unknown	348.95	273.20	180.29	104.19
SRV_07376_at	Unknown	LU	NUU	Unknown	57.64	67.15	92.23	141.45
SRV_07501_at	Unknown	QLCU	UCC	Unknown	71.59	113.49	146.49	179.79
SRV_07809_at	Unknown	LU	NUC	Unknown	423.83	541.54	668.65	808.22
SRV_07933_at	Unknown	LU	NUC	Unknown	268.13	300.18	419.36	422.18
SRV_08376_at	Unknown	QLCD	NND	Unknown	4,406.43	4,077.72	3,023.17	2,214.33
SRV_08476_at	Unknown	LD	NDC	Unknown	411.45	351.27	222.65	150.64
SRV_08599_a_at	Unknown	LU	UCU	Unknown	50.74	79.71	105.12	187.60
SRV_08811_at	Unknown	LD	NND	Unknown	2,605.47	2,211.77	1,806.50	1,484.89
SRV_08912_a_at	Unknown	LD	NDC	Unknown	233.71	189.65	130.93	113.35
SRV_09165_at	Unknown	LD	NDC	Unknown	376.10	256.62	219.41	185.14
SRV_09248_at	Unknown	LD	NND	Unknown	1,285.44	1,086.55	870.91	679.19
SRV_09326_s_at	Unknown	QLVU	NUU	Unknown	278.11	335.24	485.81	1,088.42
SRV_09327_a_at	Unknown	LD	NDC	Unknown	370.27	287.36	195.86	169.20
SRV_09415_at	Unknown	LU	NNU	Unknown	133.42	151.32	185.15	231.10
SRV_09416_at	Unknown	LU	NNU	Unknown	1,141.63	1,283.77	1,558.44	1,844.29
SRV_09427_at	Unknown	LD	NND	Unknown	234.29	218.15	164.46	120.34
SRV_09459_at	Unknown	QLCD	NDD	Unknown	1,036.15	768.44	470.92	255.20
SRV_09599_at	Unknown	LU	NNU	Unknown	8,827.22	8,639.92	11,040.88	13,897.56
SRV_09788_s_at	Unknown	LU	NNU	Unknown	795.41	933.33	1,149.29	1,408.13
SRV_09891_at	Unknown	QLVD	NDC	Unknown	235.98	163.69	143.54	144.52
SRV_09914_x_at	Unknown	LD	NDC	Unknown	233.72	173.49	144.97	111.06
SRV_09951_at	Unknown	LU	NNU	Unknown	191.49	188.17	274.78	376.91
SRV_10204_at	Unknown	LD	NND	Unknown	741.08	621.67	510.89	385.84
SRV_10498_at	Unknown	LU	NUC	Unknown	7,379.03	7,708.85	11,198.04	13,043.79
SRV_05456_a_at	USP2	LU	NNU	Protein binding	315.37	348.44	362.86	555.99
SRV_02868_at	VARS	LD	NND	Defense response	304.30	267.66	234.39	200.54

SRV_05093_at	VIT	LU	NNU	Unknown	654.21	726.32	886.76	1,042.90
SRV_03854_a_at	VPS24	LU	NNU	Protein binding	279.00	351.85	400.99	441.91
SRV_01863_at	VRK1	LD	NDC	Protein binding	650.03	439.78	343.72	262.01
SRV_05528_at	ZADH2	LU	NNU	Zinc ion binding	295.36	364.61	404.19	448.61
SRV_02759_at	ZFAND5	QLCU	UCC	Ion binding	218.22	372.59	465.18	455.28

Probe-set ID represents the unique identifier for each probe-set on the custom *Ambystoma* GeneChip. The column "Pattern" describes the gene expression patterns on days 56, 70, and 84 post hatching and the column "Model" describes the regression models fit to the gene expression patterns on days 42, 56, 70, and 84 post hatching for genes uniquely expressed by ≥ 1.5 -fold in *A. t. tigrinum*. The columns denoted by "42 DPH", "56 DPH", "70 DPH", and "84 DPH" refer to the mean fold change observed on days 42, 56, 70 and 84 post hatching.

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